Canadian Journal of Research

Issued by THE NATIONAL RESEARCH COUNCIL *of* CANADA

VOLUME 5 OCTOBER, 1931 NUMBER 4

PREPARATION AND HEAT DENATURATION OF THE GLUTEN PROTEINS[1]

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Abstract

Gliadin prepared by several different methods had the same nitrogen content and distribution. The critical peptization temperature (C.P.T.) in 60% alcohol and viscosity in 30% urea-buffer solutions, however, showed considerable variation, preparations of high C.P.T. (low solubility) being more viscous. This variation in the physical properties is explained by fractionation or denaturation incidental to the method of preparation.

Gluten precipitated from 30% urea solutions at salt concentrations varying from 0.1 to 0.5 of saturation, yielded fractions that varied continuously in their gliadin and glutenin content, as judged from their percentage of arginine nitrogen.

Gluten dispersed in buffered 30% urea solutions showed no change in viscosity during 101 hr. after the gluten was completely dispersed. A variation of hydrogen ion concentration between pH 6.0 and 6.95 had little effect on its viscosity. Heating at 70° C. caused a marked decrease in the viscosity of this dispersion during the first hour. When gliadin dispersions are heated as above only samples having a high initial viscosity and C.P.T. become less viscous. Heating gliadin of natural moisture content (12 to 14%) at 70° C. for varying periods of time did not change significantly its subsequent C.P.T. and viscosity in 60% alcohol. More severe heat treatments at higher moisture contents rendered the gliadin insoluble in 60% alcohol. Dilute alcoholic extracts of heated flours contained less protein than those of unheated controls. However, the C.P.T. of the former was lower than that of the latter. It is concluded from these experiments that when the gluten proteins are subjected to elevated temperatures, the glutenin fraction is first affected, next the gliadin fractions of low solubility, and finally, under severe conditions, all of the gliadin is denatured.

Introduction

The available data on the heat denaturation of the gluten proteins have been obtained largely from investigations conducted on either flour or gluten. This has been inevitable since the gliadin and glutenin, which make up gluten, have been difficult to isolate and study without altering their colloidal properties. Recent experiments have shown, however, that the changes induced in these

[1] *Manuscript received July 31, 1931.*

Contribution from the Food Research Institute and Department of Chemistry, Stanford University, California, and the Department of Field Crops, University of Alberta, with financial assistance from the National Research Council of Canada. The present paper is being issued as Paper No. 26 of the Associate Committee on Grain Research. It constitutes part of a thesis presented to the Department of Chemistry, Stanford University, in partial fulfilment of the requirements for the degree of Doctor of Philosophy.

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proteins by elevated temperatures are of commercial as well as theoretical importance, and this has stimulated research along these lines. The present investigation was conducted to determine how the two gluten proteins were affected by heat treatment.

The commercial value of heat-treated flour has been demonstrated recently by Kent-Jones (16) who found that the baking quality was improved by controlled heat treatment. Moreover, he has found that an excessively heat-treated flour, although ruined for bread making purposes when used alone, may be used in small amounts as a flour improver. Both Kent-Jones (16) and Herd (13) conclude that some alteration of the protein is responsible, not only for the decreased baking quality of the excessively heat-treated flour when used alone, but also for the increased baking quality when such a flour is added to other flours as an improver.

An extensive investigation on the drying of wheat, conducted by the National Research Council of Canada (17) showed that wheat decreases in quality when the temperature of the air used for drying exceeds 180° F. At higher air temperatures the decrease in quality is roughly proportional to the temperature increase.

Geddes (7, 8) has repeated part of the investigations carried out by Kent-Jones. Under certain conditions of heating an improvement in baking quality was observed, while more excessive heat treatments resulted in a corresponding decrease in baking quality. He found that the extent and rate of swelling of the gluten washed from these flours, and the viscosity of acidulated flour-in-water suspensions decreased with increasing severity of heat treatment. Geddes also determined the amount of protein dispersed by a normal solution of magnesium sulphate and potassium iodide by the method of Gortner, Hoffman and Sinclair (9), and found that the flour proteins became progressively less soluble with increasing amounts of heat treatment. He concluded that the effect of heat on the gluten proteins is always detrimental to baking quality, and that the improvement observed after mild heat treatments is probably due to the effect of heat on the lipoid fraction of the flour.

The temperature at which the gluten is altered depends on the moisture content and time of heating. Kent-Jones (16) found that it was necessary to heat flour of "natural moisture content" for 20 hr. at 130° F. (54.4° C.) to produce a change similar to that obtained by two hours' heating at 150° F. (65.6° C.). Geddes' results (7) show that six to eight hours' heating at 130° F. (54.5° C.) are required to produce a significant change in the baking quality of a flour containing 13.9% moisture. Geddes (7, 8) has shown that the effect of heat on flour, as judged by its subsequent baking quality and protein solubility, increases with its moisture content. This is in harmony with the earlier work of Chick and Martin (4), who found that moist egg albumin could be subjected to temperatures of 120° C. for several hours before any appreciable denaturation took place, provided the moisture was allowed to evaporate during the treatment. If, however, the protein were placed in a sealed tube (*i.e.*, in the presence of steam), complete denaturation occurred at this temperature in a few moments.

Alsberg and Griffing (1) found that the swelling of gluten disks, subjected to 30 min. heating at various temperatures from 50° to 80° C., decreased as the temperature increased. The rate of decrease in swelling power was greatest between 60° and 65° C., and with this exception these workers found no indication of a definite coagulation temperature. This appears to be in agreement with the work of Chick and Martin (4) on egg albumin; these writers do not regard coagulation as taking place at a definite temperature, but rather consider that this process is a chemical reaction with a very high temperature coefficient. At temperatures between 30° and 50° C., Alsberg and Griffing found no decrease in swelling power, in fact, they obtained some evidence of an increased swelling.

There is little information as to the effect of heat on the individual gluten proteins except that which has been inferred from indirect evidence. Purified glutenin has apparently never been studied from this standpoint, although it is known that a dispersion of glutenin in dilute alkali is not coagulated on heating, thus differing from egg albumin in its reaction to heat. Gottenberg and Alsberg (10) found that gliadin dispersed in 60% alcohol was not seriously altered in solubility when held at a temperature of 58° C. for 15 days. This indicates that either it is not affected by being heated to this temperature, or that the 60% alcohol protected it from denaturation. This latter possibility is suggested by the fact that gliadin dispersed in 20% alcohol showed a significant increase in its C.P.T. on being heated.

Experimental

Methods of Analysis

The methods employed for determining the moisture and nitrogen contents and the nitrogen distribution were the same as those described in an earlier paper by Cook and Alsberg (5).

Hydrogen ion concentration. This determination was made with a quinhydrone electrode at 25° C.

Viscosity. All viscosity measurements were made in Ostwald viscometers. These were calibrated with water and sucrose solutions, using the values given in the International Critical Tables (15, p. 23). The measurements were made in a thermostat at 25° C. ±0.05, the specific gravity determinations being carried out at the same temperature.

Critical peptization temperature. This has been defined by Dill and Alsberg (6) as the temperature at which turbidity appears when a solution of gliadin in alcohol-water is cooled. On the other hand, if the gliadin is originally turbid in dilute alcohol at ordinary temperatures, this turbidity will usually clear up at a definite temperature which can be observed if the dispersion is heated slowly. The temperature at which the solutions became clear on heating, and the temperature at which they again became turbid on cooling were averaged to obtain the reported values. The technique employed was that described by Dill and Alsberg.

Preparation and Properties of Gliadin

Before studying the heat denaturation of gliadin, some attention was given to the preparation of this protein. Three methods described in the literature were employed in preparing samples. One of these methods yielded three fractions. Another preparation was made using urea solution as the dispersion medium. A description of these preparations follows. Only the procedure which yielded different fractions, and the urea method, are described in detail.

Preparation 1. This sample of gliadin was prepared by extraction with 70% alcohol in essentially the same manner as that described by Osborne (18).

Preparation 2. This sample was prepared by extraction with 60% alcohol and purified by the method of Dill and Alsberg (6). This procedure is essentially that of Osborne, using additional precautions to obtain purity and prevent denaturation.

Preparations 3 and 4. These preparations were obtained from an acetic-acid extract of dried gluten, using the method of Blish and Sandstedt (2). The 18 litres of 0.07 *N* acetic acid, containing the dispersed gliadin from the initial extraction, was filtered through paper pulp and 200 gm. of lithium chloride added to precipitate the gliadin. The solution was stirred vigorously while adding the salt, in order to precipitate the gliadin as a foam which is easy to wash. The foam was then removed and allowed to stand at 2.5° C. overnight and the drainage water removed. It was then adjusted to a 60% alcohol concentration and again allowed to stand at 2.5° C., which gave a precipitate and a clear supernatant liquid, which was concentrated at low pressure and below 50° C. to about one-fourth its original volume. On standing at 2.5° C., this solution did not give a precipitate, so it was precipitated by pouring it into 1% lithium chloride solution and purified separately. On purification by one aqueous and one alcoholic precipitation, this gave Preparation 3. The precipitate obtained at 2.5° C. was unfortunately lost by accident in dehydrating the material. Preparation 4 was obtained from the original acetic acid dispersion. After removing the foam, this liquid was quite turbid, so it was allowed to stand overnight at 0° C. The resulting precipitate did not give a clear solution in 60% alcohol, and only a small amount of material settled out on standing. After discarding this precipitate, the dispersion, though opaque, appeared to be quite homogeneous at room temperature. It was consequently purified by one aqueous and one alcoholic precipitation, and dehydrated.

Preparation 5. This preparation was obtained by dispersing freshly washed gluten in 30% urea, supercentrifuging to remove the starch, and then precipitating the glutenin at 0.18 of saturation with magnesium sulphate. The precipitated glutenin was removed by supercentrifuging, and 500 gm. of solid magnesium sulphate added to two litres of the liquid so obtained. The clear liquid immediately became milky but as no precipitate settled out on standing 24 hr. at 0° C., it was removed by supercentrifuging. The liquid which was removed still contained considerable protein, but it was discarded. Since gliadin is dispersed by a 10% urea solution (5), the precipitate from the supercentrifuge was placed in a urea solution of this concentration in which it gave

an opaque dispersion. Addition of magnesium sulphate to 0.12 of saturation precipitated most of the protein. On redispersing the protein in 10% urea, and supercentrifuging to remove the denatured material, a clear solution was obtained. This was poured into five volumes of water and allowed to stand 24 hr. to settle. The precipitate was then washed and dehydrated with alcohol and ether. This preparation was dispersed by 60% alcohol, but the dispersion was more opaque than that of gliadin prepared by the alcohol method.

The nitrogen content and distribution of these preparations are given in Table I. Preparations 1, 2, 3 and 5, isolated by different methods, appear to have essentially the same composition. Preparation 4 has a lower total nitrogen content, and a higher percentage of amide nitrogen, for which there is no apparent explanation. This preparation also has a significantly lower arginine content. Owing to the peculiar solubility of this sample, and its difference in composition, it was not included in the average.

TABLE I

NITROGEN CONTENT AND DISTRIBUTION OF GLIADIN PREPARATIONS

Preparation No.	Method of preparation	Nitrogen content	Nitrogen distribution								
			Humin N	Amide N	Arginine N	Phosphotungstic acid precipitation					Recovery
						Arginine N in filtrate	Values for fractions corrected for arginine nitrogen in filtrate				
							Basic N	Total filtrate N	Filtrate amino N	Filtrate non-amino N	
		%	%	%	%	%	%	%	%	%	%
1	Osborne (18)	17.30	0.006	25.4	5.67	3.21	10.4	62.7	54.4	8.3	98.5
2	Dill and Alsberg (6)	17.48	0.001	26.0	5.81	3.31	12.0	62.0	55.0	7.0	100.0
3	Blish and Sandstedt (2)	17.71	0.007	26.1	5.76	2.88	9.4	62.8	53.3	9.5	98.3
4	Blish and Sandstedt (2)	17.24	0.001	28.0	5.19	2.64	10.0	61.6	53.3	8.3	99.6
5	Urea dispersion	17.57	0.040	26.6	5.80	3.20	10.8	63.6	56.1	7.5	101.0
Average of preparations No. 1, 2, 3 and 5		17.50	—	26.0	5.76	—	10.6	62.8	54.7	8.1	—

The physical properties of these preparations, however, are quite different. Their critical peptization temperatures, (C.P.T.), viscosities of 5% dispersions in 60% alcohol (by volume), and viscosities of 4% dispersions in buffered 30% urea solutions are given in Table II.

In this table the gliadin samples have been arranged in the order of increasing C.P.T. It was impossible to get a sharp reading for the C.P.T. of Preparations 4 and 5, but the dispersions did clear up in the region of the reported temperatures. It is evident that there is a relationship between the C.P.T. and the viscosity of the dispersions, those of high C.P.T. being more viscous.

TABLE II

CRITICAL PEPTIZATION TEMPERATURE AND VISCOSITY OF GLIADIN PREPARATIONS

Preparation No.	Method of preparation	C.P.T. °C.	Gliadin (5%) in 60% alcohol, centipoises	Gliadin (4%) in 30% urea-buffer, centipoises
3	Blish and Sandstedt	−7.4*	—	2.64
1	Osborne	7.6	5.30	2.59
2	Dill and Alsberg	12.4	5.90	3.05
5	Urea-dispersion	35–40**	—	3.24
4	Blish and Sandstedt	80–90**	—	5.91

**This low C.P.T. can be attributed partly to some acetic acid having been retained by the protein (See Table VIII).*

***No definite temperature.*

These results show that although different samples of gliadin prepared from the same flour, by various methods, have the same chemical composition, they differ considerably in their physical properties. This can be explained either by a fractionation of the original material, or by a partial denaturation of the gliadin.

The first explanation appears to be the correct one, judging from the recent work of Haugaard and Johnson (12). These workers have shown that gliadin can be divided into a number of fractions differing in solubility, although almost the same in chemical composition. They have also shown that each portion so obtained can be fractionated further, again yielding one portion of lower and another of higher solubility than the original. This provides an explanation for the low C.P.T. of Preparation 3 and the high C.P.T. of Preparation 4 which were both obtained from the same gluten extract. It also explains the relatively high C.P.T. of the preparation from urea, for only part of the gliadin was precipitated from the supernatant liquid, and presumably this represented the more insoluble fractions. Haugaard and Johnson also found that fractions of low maximum solubility had higher viscosities and C.P.T.'s than those of high maximum solubility, a finding in agreement with the above results.

The possibility of some denaturation having taken place in these gliadin preparations cannot be entirely excluded. Although the different physical properties can be explained by the extraction of different fractions, it was found that these samples all gave a positive test for a thiol group when tested with sodium nitroprusside. The work of Harris (11) and Hopkins (14) has shown that with such proteins as egg albumin this reaction can be taken as an index of denaturation. It may be, however, that gliadin gives this reaction originally although it seems probable that it does not (5). The urea preparation showed no significant difference from the others in this respect.

FRACTIONATION OF GLUTEN

Glutenin is a more difficult protein to study than gliadin from the standpoint of heat denaturation. Although a method has been developed for

preparing this protein in neutral solution, it has so far been found impossible to obtain glutenin in a dry form without denaturing it. A study of the heat denaturation of glutenin must therefore be confined to dispersions.

The possibility of glutenin not being a definite entity complicates its study still further. Blish and Sandstedt (3) conclude that there is insufficient evidence to show that glutenin is a chemical individual. The similar composition of different glutenin samples prepared from the same flour by the urea method (5) makes the possibility of different chemical fractions seem unlikely. Glutenin may, however, be separable like gliadin into a number of fractions which have different physical properties, though almost identical chemically. Although no definite evidence of this has yet been obtained it was observed in preparing glutenin by the urea method that this protein was not completely precipitated, as judged by the opaque supernatant fluid, either by the addition of salt or water, until sufficient had been added to come just within the lower precipitation limit for gliadin. Furthermore, the precipitation of gluten from a urea dispersion appeared to be a continuous process, for after removing the first precipitate, a further addition of salt or water would yield another precipitate. To obtain further information on this point a sample of gluten was fractionated in the following manner:

A dispersion of gluten was prepared by placing 200 gm. of wet gluten in 1500 cc. of 30% urea solution at pH 6.9. After standing overnight the dispersion was supercentrifuged to remove the starch. Saturated magnesium sulphate in 30% urea solution was then added until the dispersion had a salt concentration of 0.13 of saturation. This was allowed to stand $1\frac{1}{2}$ hr., supercentrifuged to remove the precipitated protein, and the salt concentration of the liquid adjusted to 0.16 of saturation. The process was then repeated, and fractions obtained at salt concentrations corresponding to 0.13, 0.16, 0.18, 0.21 and 0.27 of saturation. Solid magnesium sulphate was then added to the solution until it was approximately half saturated. This made the liquid turbid, but some difficulty was encountered in getting this material to settle. Finally, it was accomplished by adjusting the hydrogen ion concentration to about pH 5.5 by the addition of a little dilute acetic acid. The complete solubility of this fraction in 60% alcohol proved it to be gliadin, so no further effort was made to recover the protein which remained dispersed. As the further purification of these samples had to be accomplished without dispersing them, since this was found in an earlier experiment to result in a further fractionation, a uniform procedure was employed throughout. All the fractions were washed eight times with ice-cold 1% lithium chloride solution. This removed most of the urea and yet prevented the partial dispersion of the more soluble fractions. These washings, however, were not very effective, as the precipitates came down in a very coherent form and had to be torn up several times during these extractions. The samples were then treated with 95% alcohol, ground up in a mortar, dehydrated with absolute alcohol and ether, and finally dried to constant weight. The nitrogen content and distribution of these fractions are given in Table III.

TABLE III

COMPOSITION OF FRACTIONS PRECIPITATED FROM A 30% UREA DISPERSION OF GLUTEN AT DIFFERENT SALT CONCENTRATIONS

Fraction No.	Concentration of $MgSO_4$ expressed in terms of saturation	Weight of protein obtained, approx., gm.	Nitrogen content, %	Humin N %	Amide N %	Arginine N %	Basic N %	Arginine N as % of non-amide N, %	Approximate proportion of each protein in fractions: Gliadin, %	Glutenin, %
1	0.13	0.2	15.9							
2	0.16	0.8	16.8	1.0	28.9	8.2		11.4	18	82
3	0.18	4.0	17.3	0.2	25.7	8.0	13.9	10.8	32	68
4	0.21	5.5	17.3	0.1	23.8	7.6	13.0	10.0	50	50
5	0.27	5.5	17.4	0.1	26.1	6.7	11.1	9.1	70	30
6	Approx. 0.5	2.0	16.7	0.2	33.0	5.8		8.7	80	20
Gliadin*					25.8	5.8		7.8		
Glutenin**					19.5	9.8		12.2		

**Average from Table I.*

***Average values for urea preparations of glutenin given by Cook and Alsberg (5).*

It is evident, from the weights of the different fractions obtained, that not more than a third of the original gluten was recovered. The reported values are by no means quantitative, however, since some loss of each fraction occurred in removing the protein from the centrifuge bowl, and in subsequent manipulations. Further, the yield reported for Fraction 6 represents only a part of the protein remaining in solution after the precipitation of Fraction 5. The magnitude and irregularity of the amide nitrogen values indicate that some urea remained sorbed on the protein, as was to be expected. The total nitrogen content reported is therefore too high. Fraction 1, however, appears to have contained some non-nitrogenous impurities. The arginine and basic nitrogen contents of these fractions, however, show a steady decrease as the salt concentration required to precipitate the fraction increases. As gliadin and glutenin contain practically the same amount of the other forms of nitrogen, no progressive change could be expected.

The progressive decrease in the arginine content of the fractions can be attributed to their containing different proportions of gliadin and glutenin. As the presence of urea in the preparations causes an error in the arginine values when expressed as a percentage of the total nitrogen, they were recalculated as a percentage of the non-amide nitrogen. These, and similar values calculated from the arginine nitrogen content of pure gliadin and glutenin, were used to estimate the amount of the two gluten proteins in each of the fractions. The computed values appear in the last two columns of Table III. It is evident from these figures that the precipitate obtained at the lowest salt concentration contains the highest percentage of glutenin, and, in succeeding precipitates, the amount of gliadin increases almost linearly with the salt concentration used for precipitation up to 0.27 of saturation. The precipitate obtained at half saturation still contained glutenin according to the analysis.

It is likely that the protein which remained in solution above this salt concentration was largely gliadin.

In the earlier paper (5) it was concluded that three precipitations were sufficient for removing the gliadin. The data given in Table III bear out this conclusion. The precipitate obtained at 0.16 of saturation contained about 18% of gliadin. This precipitate was not washed with water. Assuming the same degree of separation in two subsequent precipitations, this would reduce the gliadin content of the final precipitate to about 2%. Furthermore, the washing incidental to each precipitation would remove still more of the gliadin.

The occurrence of glutenin in the precipitates obtained at high salt concentrations can be explained by assuming: (a) that this protein is similar to gliadin in that it consists of a number of fractions of different solubilities; or (b) that there is an intimate relationship between the two proteins, the more insoluble glutenin being protected from precipitation by the gliadin. The effect of heat treatment on a whole gluten dispersion was consequently undertaken before attempting similar studies on its component parts.

The Heat Denaturation of Gluten Dispersions

A gluten dispersion was prepared from 170 gm. of wet gluten, 30 gm. of solid urea, and 700 cc. of neutral 30% urea solution. This was allowed to stand for 36 hr. at 0° C. to permit complete dispersion before passing it through a Sharples supercentrifuge to remove the starch. As urea solutions tend to become alkaline on being heated, it was necessary to add a suitable buffer to the solution. Potassium dihydrogen phosphate—disodium hydrogen phosphate ($KH_2PO_4 - Na_2HPO_4.12H_2O$) mixtures were chosen for this purpose. As different samples of urea vary somewhat in their reaction, the two components of the buffer system were made up separately in double concentration in 30% urea solution, and these mixed to give a pH of 6.85, as determined experimentally with a quinhydrone electrode. Equal volumes of the protein dispersion and the combined buffer were then mixed. This procedure diluted both the buffer solution and the protein dispersion to one-half their original concentration.

It was not necessary that the exact protein concentration of the dispersion be known, since the effect of any particular treatment could be determined only from the relative values observed. However, attempts were made to determine the amount of protein in dispersion by comparing its solid content with that of the dispersion medium. The results indicated a protein concentration of about 3%, but it was found difficult to check these determinations accurately owing to the large solid content of the solution as compared with the amount of protein it contained.

The effect of storage and hydrogen ion concentration on the viscosity of the above gluten dispersion was studied before investigating the effect of heat treatment.

In the first experiment the hydrogen ion concentration after mixing the protein and buffer solutions was equal to 6.95 pH. Viscosity determinations

were made on this dispersion immediately and at various intervals during storage at 5° C. It should be noted that about 40 hr. had elapsed from the time the gluten was first placed in the urea solution until this experiment was started. The results, which are presented in Table IV, confirm the earlier observations (5) that neither the hydrogen ion concentration nor the viscosity of the dispersion change on being stored, for periods up to 101 hr. Apparently the urea solution causes no further change in the protein after complete dispersion has taken place.

TABLE IV

EFFECT OF TIME OF STORAGE ON THE VISCOSITY OF AN APPROXIMATELY 3% GLUTEN DISPERSION IN 30% UREA-BUFFER SOLUTION*

Time of storage at 5° C., hr.	pH	Viscosity, centipoises	Time of storage at 5° C., hr.	pH	Viscosity, centipoises
Initial	6.95	3.38	27.5	7.00	3.28
4.0	—	3.34	51.5	6.95	3.32
10.0	6.95	3.29	101.0	6.95	3.34

**The gluten had been placed in the urea solution about 40 hr. before this experiment was started, to permit complete dispersion of the protein.*

The effect of hydrogen ion concentration was studied only in the region of neutrality. The composition, pH and viscosity of the protein dispersions used are given in Table V. The results indicate a slight decrease in viscosity as the reaction becomes more alkaline, but there is no evidence of an isoelectric point within the range studied. Since the dispersion medium had a different composition for each hydrogen ion concentration, viscosity determinations were also made on identical solutions without dispersed protein. These values showed practically no change, varying only from 1.19 centipoises at pH 6.00 to 1.20 centipoises at pH 7.35.

TABLE V

EFFECT OF HYDROGEN ION CONCENTRATION ON THE VISCOSITY OF AN APPROXIMATELY 3% GLUTEN DISPERSION IN DIFFERENT 30% UREA-BUFFER SOLUTIONS

Composition of dispersion			pH	Viscosity of final dispersion 1+2+3, centipoises
Solution 1 (9.078 gm. KH_2PO_4 in 500 cc. 30% urea), cc.	Solution 2 (11.876 gm. $Na_2HPO_4.12H_2O$ in 500 cc. 30% urea), cc.	Solution 3 (Approximately 6% dispersion of gluten in 30% urea),* cc.		
48.75	1.25	50.00	6.00	3.44
47.50	2.50	50.00	6.00	3.44
40.00	10.00	50.00	6.35	3.45
30.00	20.00	50.00	6.65	3.42
20.00	30.00	50.00	6.95	3.34
10.00	40.00	50.00	7.35	3.28

**The protein concentration in the final dispersion 1+2+3 would, therefore, be approximately 3%.*

The influence of heating on the viscosity of this gluten dispersion was determined by heating it in long narrow test tubes, which were stoppered tightly, and placed in a water bath at $70 \pm 0.5°$ C. for varying periods of time. The results obtained are presented in Table VI. It is evident that the viscosity falls off very rapidly during the first hour of heating. After that, a further, but much slower, decrease in viscosity results. Judging from the last experiment on the effect of hydrogen ion concentration, it seems probable that the decrease in viscosity after the first hour is due to the dispersion becoming more alkaline. To give a better picture of these results, the viscosity of the dispersion has been plotted against the time of heating and the resulting chart is shown in Fig. 1.

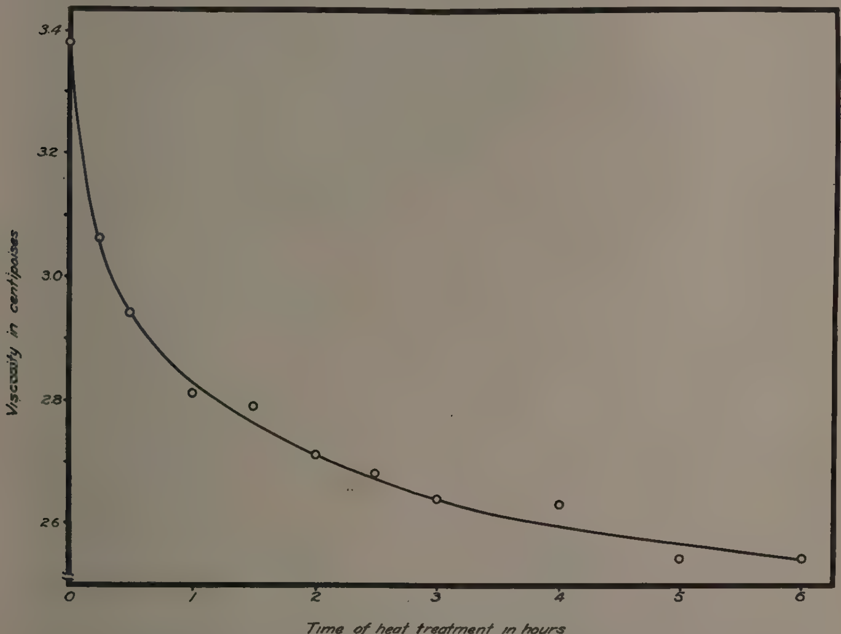

FIG. 1. *The influence of the time of heating at 70°C. on the viscosity of an approximately 3% dispersion of gluten in buffered 30% urea solution.*

TABLE VI

EFFECT OF HEAT TREATMENT ON THE VISCOSITY OF AN APPROXIMATELY 3% GLUTEN DISPERSION IN 30% UREA-BUFFER SOLUTION

Time of heat treatment at 70° C.		pH	Viscosity, centipoises	Time of heat treatment at 70° C.		pH	Viscosity, centipoises
hr.	min.			hr.	min.		
Not heated		6.95	3.38	2	30	7.20	2.68
0	15	7.00	3.06	3	00	7.35	2.64
0	30	6.95	2.94	4	00	7.45	2.63
1	00	7.00	2.81	5	00	7.65	2.54
1	30	7.05	2.79	6	00	7.75	2.54
2	00	7.10	2.71				

Heat Denaturation of Gliadin

Having established a definite decrease in the viscosity of gluten dispersions heated at 70° C., the investigation was continued to determine the effect of various heat treatments on the properties of purified gliadin. This protein was heated in two forms: (a) solid, of natural moisture content, since this approximates the conditions under which the baking quality of flour is altered; and (b) dispersed in urea-buffer solutions, to provide data comparable with those obtained for gluten dispersions. The gliadin preparations described earlier provided experimental material. The heat treatment of an alcoholic dispersion of gliadin was not undertaken, since the results of Gottenberg and Alsberg (10) indicate that gliadin dispersed in 60% alcohol was not significantly altered by being held at 58° C.

The plan of experiment followed with the solid material was to heat, dry, and finally disperse the gliadin in 60% alcohol. The C.P.T. and viscosity of the resultant dispersion were taken as criteria of change. Preliminary experiments had shown that the protein could not be completely dispersed if it had been subjected to temperatures over 80° C. Samples of high moisture content were rendered insoluble at even lower temperatures. Subsequent experiments were therefore confined to samples of natural moisture content (12 to 14%) at a temperature of 70° C.

The procedure followed was to weigh accurately into weighing bottles samples of gliadin somewhat in excess of one-half gram. The bottles were then sealed with a hard cement, and the samples heated in a water bath for various periods at $70 \pm 0.5°$ C. On removal from the bath, the bottles were again weighed to determine if any loss in moisture had taken place, opened, and the gliadin samples dried over phosphorus pentoxide *in vacuo* at room temperature. When the samples had reached constant weight, the weight of protein in the bottles was adjusted accurately to 0.5000 gm. and 5 cc. of 60% alcohol added to each bottle. The stoppers were then replaced and sealed, and the gliadin allowed to disperse for three days at room temperature with occasional shaking. It was observed that the samples exposed to the longest heat treatments dispersed more slowly than the others. When all the samples were completely dispersed, the volume was adjusted to exactly 10 cc. by the addition of 60% alcohol, allowed to stand for another day, and samples taken for viscosity and C.P.T. measurements. The results obtained are given in Table VII, each value reported being the average of at least duplicate determinations.

It is evident from the moisture data that the samples dried out somewhat while being heated. The C.P.T., however, shows no significant change for either preparation, although the regular and progressive increase in the temperatures for Preparation 1 suggests that these samples may have become slightly less soluble. The viscosity determinations are somewhat irregular, a result which must be attributed to error as no progressive change is evident. The conclusion to be drawn from this experiment is that gliadin is not significantly altered in C.P.T. or viscosity by being heated at 70° C. for several hours at its natural moisture content.

TABLE VII

EFFECT OF HEAT-TREATING GLIADIN OF NORMAL MOISTURE CONTENT ON ITS SUBSEQUENT C.P.T. AND VISCOSITY IN 60% ALCOHOL

Time of treatment at 70° C. hr. min.	Moisture content			C.P.T. 5% solution, °C.	Viscosity 5% solution, centipoises
	Before heating %	After heating %	Average %		
Preparation No. 1					
Not heated	14.3	14.0	14.2	8.6	5.11
Not heated	14.0	13.8	13.9	8.4	5.47
0 15	14.1	13.9	14.0	8.8	5.22
0 30	14.2	14.0	14.1	9.0	—
1 00	14.0	13.8	13.9	9.1	5.31
1 30	14.0	13.5	13.8	9.2	—
2 00	14.0	13.4	13.7	9.4	5.30
2 30	14.1	13.4	13.8	9.6	5.46
4 00	14.0	12.8	13.4	9.8	5.30
5 00	13.9	12.4	13.2	9.6	5.57
6 00	14.0	12.4	13.2	9.6	—
Preparation No. 2					
Not heated	13.2	12.7	13.0	10.7	5.95
Not heated	12.5	12.3	12.4	10.9	5.86
0 15	12.8	12.6	12.6	11.2	6.05
0 30	12.9	12.5	12.7	11.1	6.21
1 00	14.7*	11.4	13.0*	11.0	6.03
1 30	13.4	12.4	12.9	11.1	6.03
2 00	13.3	10.2	11.8	11.3	6.06
2 30	12.8	11.7	12.2	11.0	6.17
3 00	14.3	11.3	12.8	11.0	5.97
4 00	12.4	11.9	12.2	11.2	6.01
5 00	12.2	11.2	11.7	11.3	6.27
6 00	13.7	9.4	11.6	11.0	6.10

**Doubtful values.*

In order to obtain information comparable with that obtained for whole gluten, 4% dispersions of gliadin were prepared in buffered 30% urea solutions. The two components of the buffer system were made up separately, and mixed to give a pH of 6.85. The protein dissolved within two hours, but the dispersions were allowed to stand for 36 hr. at 0° C. before being heated. They were then transferred to long, narrow test tubes, which were stoppered tightly and put in a water bath at $70 \pm 0.5°$ C. for the required length of time. When removed from the bath, the tubes were cooled quickly and the pH and viscosity of the protein dispersions determined immediately. The average values for duplicate determinations are given in Table VIII.

The results show that the dispersion became more alkaline as the time of heat treatment increased, but this change was usually small during the first $1\frac{1}{2}$ to 2 hours. The dispersion of Preparation 3 was originally much more acidic than the others, and as the buffer solution originally had a

TABLE VIII

EFFECT OF HEAT TREATMENT ON THE VISCOSITY OF 4% GLIADIN DISPERSIONS IN 30% UREA SOLUTIONS

Time of treatment at 70° C. hr.	min.	pH	Viscosity, centipoises
Preparation No. 1			
Not heated		6.90	2.59
0	30	6.95	2.52
1	30	7.10	2.54
2	30	7.35	2.58
Preparation No. 2			
Not heated		6.95	3.05
0	15	6.90	2.97
0	30	6.95	2.88
1	00	7.00	2.92
2	00	7.15	2.87
3	00	7.35	2.79
4	25	7.90	2.84
Preparation No. 3			
Not heated		6.45	2.64
0	15	6.45	2.63
0	30	6.50	2.60
1	00	6.70	2.66
1	30	6.85	2.58
2	00	6.95	2.58
2	30	7.10	2.58
3	00	7.15	2.61
4	00	7.35	2.58
5	00	7.55	2.56
6	00	7.80	2.61
Preparation No. 4			
Not heated		6.85	5.91
1	30	7.05	4.71
Preparation No. 5			
Not heated		6.90	3.24
1	30	7.05	3.07
Dispersion medium without protein			
Not heated		—	1.18
6	00	—	1.22

pH of 6.85, this change must be attributed to the protein. The only apparent reason for this acidic property is that some acetic acid remained sorbed on the gliadin, from its initial extraction with the solvent.

The viscosity of Preparations 1 and 3 was apparently unaltered by heat. Dispersions of the remaining preparations, however, showed a decrease in viscosity after being heated. Only one period of heat treatment was possible with Preparations 4 and 5 owing to lack of material. The results given at the foot of the table show the initial viscosity of the dispersion medium and its viscosity after six hours' heating at 70° C. This treatment increased the viscosity somewhat, but not sufficiently to affect the results obtained with the protein dispersions.

The decrease in viscosity of the gluten and gliadin dispersions, after $1\frac{1}{2}$ hours' heating, has been expressed as a percentage in Table IX. It should be noted that the gluten dispersion and the gliadin dispersions are perhaps not strictly comparable since their concentrations are different. Nevertheless the heat treatment decreased the viscosity of the less concentrated gluten dispersion more than it did that of the gliadin dispersions with the exception of Preparation 4 which had a high initial viscosity. This indirect evidence indicates that glutenin is the component of gluten most easily altered by heat. More direct information as to the effect of heat on glutenin awaits further investigation.

The different physical properties of these gliadin preparations initially (see Table II) have been harmonized with the work of Haugaard and Johnson (12) who have shown that gliadin can be fractionated. Evidently the same considera-

TABLE IX

Decrease in viscosity of gluten and gliadin dispersions after heating at 70° C.

Material	Viscosity		Decrease	
	Initial, centipoises	After $1\frac{1}{2}$ hours' heating, centipoises	Centipoises	%
Gluten	3.38	2.79	0.59	17.4
Gliadin				
Preparation 1	2.59	2.54	0.05	1.9
Preparation 2	3.05	2.87*	0.18	5.9
Preparation 3	2.64	2.58	0.06	2.3
Preparation 4	5.91	4.71	1.20	20.3
Preparation 5	3.24	3.07	0.17	5.2

**This determination was made after two hours' heating.*

tions will explain the apparent anomalies as to the effect of heat treatment. Thus Preparations 1 and 3, having the lowest viscosities and C.P.T.'s originally, show no significant change in viscosity on being heated. The viscosities of Preparations 2, 4 and 5, however, were decreased by heat treatment, the extent of the change increasing with the initial viscosity and the C.P.T. of the sample. The more insoluble fractions of gliadin are therefore the first to be affected by heat, the magnitude of the change caused by a given heat treatment depending on the solubility of the fraction.

A further study of the heat denaturation of gliadin was made by investigating the properties of alcoholic extracts from heated flours. Samples of two flours milled from hard red spring wheat were placed in a saturated atmosphere at 0° C. until they had a moisture content of 17.9 and 19.1% respectively. Samples of approximately six grams each were then packed tightly into glass tubes of about 0.5 cm. internal diameter, the tubes sealed, and then heated for varying periods of time at 96.2° C. The small tubes were used to permit rapid and thorough heating of the whole sample. After being heated, the flour samples were transferred to tared dishes and dried at 98° C. *in vacuo*. (It will be shown later that the heat treatment incidental to drying had no effect on the properties under investigation.) Five-gram samples of the dry flour were then weighed into test tubes, 25 cc. of 60% alcohol (by volume) added, and the tubes stoppered. They were allowed to stand for 32 hr. at room temperature, and then shaken for six hours at 45° C., cooled, and the supernatant liquid removed after centrifuging. The nitrogen content and C.P.T. of the extract were then determined. The results are presented in Table X, and represent the average of duplicate heat treatments on each flour.

The results obtained were essentially the same for both flours. The irregularities in moisture content can probably be attributed to evaporation losses in transferring the flour from the tubes to the drying dishes, since the actual heat treatment was performed in sealed tubes. Comparison of the first two values for each flour shows that drying at 98° C. *in vacuo* had no effect on the

TABLE X

EFFECT OF HEAT-TREATING FLOUR ON THE AMOUNT OF GLIADIN EXTRACTED BY 60% ALCOHOL AND THE C.P.T. OF THE EXTRACT

Time of heat treatment at 96.2° C. hr.	min.	Moisture content of flour %	Protein concentration in 60% alcohol extract %	C.P.T. of extract °C.
Flour I				
Dried over $CaCl_2$ *in vacuo.* No heat treatment given		17.9	2.9	12.0
Dried at 98° C. *in vacuo* immediately		18.4	3.0	12.0
0	15	17.6	1.3	6.5
0	30	17.9	0.9	6.0
1	00	17.8	1.0	7.0
2	00	17.7	0.6	6.0
4	00	17.6	0.4	6.0
8	00	17.3	0.5	6.5
Flour II				
Dried over $CaCl_2$ *in vacuo.* No heat treatment given		19.1	2.4	11.0
Dried at 98° C. *in vacuo* immediately		19.4	2.4	11.0
0	15	18.7	0.9	6.5
0	30	19.1	0.6	6.5
1	00	18.8	0.7	6.5
2	00	18.4	0.5	6.5
4	00	18.2	0.4	6.0
8	00	18.3	0.4	6.5

properties studied. The heated samples were consequently dried at this temperature. The figures for the amount of protein extracted by the alcohol were calculated from the nitrogen content of the extract. These values are also somewhat irregular, but in general they show a progressive decrease with increased time of heating. The C.P.T. of the extracts is distinctly lower for all the heated flours. There is however no progressive change in this property with increasing time of treatment. Dill and Alsberg (6) have shown that the C.P.T. is largely independent of the gliadin concentration, but, as the concentration varied considerably in these extracts, this point was tested by adding an equal volume of 60% alcohol to those extracts which had a high C.P.T. and the determination repeated. No change in C.P.T. resulted.

Comparison with the controls shows that 15 min. heat treatment lowered the gliadin content of the extract about one-half, and decreased the C.P.T. about 5° C. This bears out the earlier conclusion that the gliadin fractions of low solubility are the first to be altered by heat. Heating the flour for longer periods caused a progressive, but less marked, decrease in the amount of protein

extracted by alcohol, but made no significant difference in the C.P.T. of the extract. The lower protein content of the extracts from the severely heated samples must therefore be attributed to incomplete extraction, probably due to occlusion by the increasingly coagulated glutenin and less soluble gliadin fractions.

Conclusions

Gliadin samples prepared by several different methods were essentially the same chemically. The solubility and viscosity of these samples, however, showed considerable variation. There are two possible explanations of this: (a) that gliadin consists of a number of fractions; or (b) that it was partly denatured in preparation and its physical properties altered. Since Haugaard and Johnson (12) have shown that gliadin can be fractionated, the first explanation is accepted. The possibility of some denaturation having occurred cannot be excluded, however, as the gliadin samples all gave a positive nitroprusside test, which may indicate denaturation if gliadin does not give this test originally. That it does seems unlikely (5).

The fractionation of gluten by precipitation from urea solutions at different salt concentrations, shows that under these conditions there is no clear cut distinction between the solubilities of glutenin and gliadin. From a chemical standpoint these fractions decreased in their arginine content with increasing salt concentration at which they were precipitated. This can be explained by the protection of the glutenin by the gliadin, the former being precipitated only when the salt concentration is sufficient to precipitate the more insoluble fractions of the latter. On the other hand, glutenin as well as gliadin may exist as a number of fractions, similar chemically but different physically.

Gliadin of natural moisture content is not altered appreciably in its C.P.T. or viscosity by being heated at 70° C., although more severe heating renders it insoluble. From the viscosity changes which occur when dispersions of whole gluten and gliadin are heated at 70° C. in urea-buffer solutions, it is concluded that glutenin is the first protein to be affected by heat, next the gliadin fractions of low solubility, and finally under severe conditions all of the gliadin may be denatured. This conclusion is supported in part by the fact that 60% alcohol extracts from heated flours contained less gliadin, but these extracts had lower C.P.T.'s than the controls, showing that the extracted gliadin had a greater solubility than that extracted from unheated flour. More direct evidence as to the effect of heat on glutenin awaits further investigation.

Acknowledgments

The author is indebted to Dr. C. L. Alsberg, Director of the Food Research Institute, Stanford University, California, for his counsel throughout the execution of this investigation; and to Dr. R. Newton, Professor of Field Crops, University of Alberta, for his ready assistance and advice.

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OCCURRENCE AND SPORULATION OF *HELMINTHOSPORIUM SATIVUM* P.K.B. IN THE SOIL[1]

By A. W. Henry[2]

Abstract

Helminthosporium sativum, one of the most prevalent fungous pathogenes affecting the roots and other parts of wheat and related grass plants, grows and fruits readily as a saprophyte on various substrata. It might therefore be expected that it would be found in abundance in the soil. Attempts to isolate it directly from field soils by plating have been successful, but only in a small percentage of the trials. Failures are apparently largely attributable to the fact that this fungus does not sporulate commonly in ordinary field soils. This has been shown by direct microscopic examination of the soil and by artificial cultures. From a study of the latter it has been found that *H. sativum* will sporulate quite readily on several different soil types including sand if they are sterilized, but not if they are not sterilized.

The fact that soils capable of supporting sporulation of this fungus may be rendered ineffective by adding small amounts of unsterilized soil, suggests that sporulation is inhibited by the saprophytic micro-organisms of the soil. It would appear that if *H. sativum* occurs in the soil, it must be present chiefly in the form of mycelium. Inability to form spores probably lessens the capability of this fungus to survive as well as to multiply in the soil.

Introduction

Although it is commonly assumed that root-rotting pathogenes of wheat and other cereals live and multiply in the soil, actually little is known regarding their saprophytic life in the soil. As such knowledge appears fundamental to an intelligent attack on root-rot problems, investigations for the purpose of accumulating further information of this sort were planned. The object of the experiments reported in this paper was to determine if *H. sativum*, one of the most prevalent and destructive of these fungous pathogenes, exists in soils which have been cropped to wheat and if so in what form.

It would seem from an examination of the literature that species of *Helminthosporium* are not particularly common in the soil. No references reporting the direct isolation of *H. sativum* from the soil have come to the writer's attention. It should be noted, however, that this species was not described until 1910 (9) and that Beckwith (1) in 1911 reported the isolation of a *Helminthosporium* from soil which had been constantly cropped to wheat for forty years. Bolley (2, 3) moreover considered *Helminthosporium* one of the genera responsible for wheat-sick soils in North Dakota. It is quite probable that *H. sativum* was the principal species encountered by these workers. In fact Drechsler (6) includes the *Helminthosporium* sp. of Beckwith and Bolley under the synonyms of *H. sativum*.

The writer (8) in 1921-22 attempted to isolate *H. sativum* directly from the soil by plating methods at St. Paul, Minnesota, but was unsuccessful. In a summary of soil fungi published in 1927 by Gilman and Abbott (7) *H. sativum*

[1] *Manuscript received July 24, 1931.*
Contribution from Department of Field Crops, University of Alberta, with financial assistance from the National Research Council of Canada.

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is not included among the species of *Helminthosporium* which had been reported up to that time as occurring in the soil. They, however, report the occurrence of three other species of *Helminthosporium* in the soil, namely *H. subulatum*, *H. interseminatum* and *H. anomalum*.

Occurrence of *H. sativum* in Alberta Soils

In order to ascertain if *H. sativum* occurs in Alberta soils, samples of the latter were first obtained from wheat fields in different parts of the province. Most of these were collected during August 1928 and were taken from fields in which the plants were still standing or in which the stubble remained. The samples were taken from between the drill rows and at the same time collections were made of adjacent wheat-culm bases. Attempts were then made in the laboratory to isolate *H. sativum* from both soil and stubble in order to determine if any relationship exists between the prevalence of this fungus on wheat and its occurrence in the soil.

In taking the soil samples, precautions were observed to avoid the introduction of the fungus from outside sources such as the air or neighboring plants. The surface soil to a depth of about an inch was removed with a sterile steel spatula. The spatula was then sterilized again by dipping in alcohol and flaming, and used for taking the soil sample and introducing it into a sterile test tube. In the laboratory the samples were prepared for plating as follows: A small thimbleful of each was worked through a wire screen ($\frac{1}{16}$ in. mesh) attached to the bottom of a one-holed cork inserted in a 200-cc. Erlenmeyer flask containing 20 cc. of sterile tap water. This apparatus had previously been sterilized at 15 lb. pressure in an autoclave. After the introduction of the soil, the flask was shaken for five minutes. Dilutions of 1-2000 and in some cases of 1-4000 were then made in other flasks containing sterilized tap water and platings from these were made after adding 1-cc. portions to tubes of acid agar as suggested by Waksman (11). Pieces of stubble or wheat bases were surface sterilized by dipping them into 70% alcohol, then placing them in a 1-1000 mercuric chloride solution for 2-3 min., and dipping them again into 70% alcohol. They were then transferred with sterile forceps to acid agar.

It will be noted from Table I, in which the results are summarized, that *H. sativum* was not obtained directly from the soil of any of the wheat fields from different parts of the province. It was, however, isolated from adjacent wheat-culm bases in about 9% of the trials.

Another series of soil samples was taken from the black soil of the Edmonton district. It was thought that this soil, being high in organic matter, might be more suitable for the growth of *H. sativum* than some of the others examined.

These samples were collected monthly over a period of more than a year from land in wheat or bearing wheat stubble and from fallow land. The results are given in Tables II and III. From Table II it is apparent that while *H. sativum* was isolated from two of the soil samples it was not obtained as often as might have been expected from its prevalence on adjacent wheat-culm bases. It was obtained for instance from only two of the soil samples as compared with nine of the adjacent stubble samples. There is of course the possibility that

TABLE I

RESULTS OF ATTEMPTS TO ISOLATE *H. sativum* FROM THE SOIL AND ADJACENT WHEAT STUBBLE COLLECTED IN DIFFERENT PARTS OF ALBERTA

No. of sample	Place collected	Date collected	Isolations of *H. sativum*			
			Soil		Stubble	
			No. of colonies	No. of plates	No. of colonies	No. of pieces stubble
45	Lacombe	Aug. 4, 1928	0	2	0	5
65	Big Valley	Aug. 6, 1928	0	3	0	6
83	De Winton	Aug. 7, 1928	0	3	1	6
91	Cayley	Aug. 8, 1928	0	2	1	6
94	Claresholm	Aug. 9, 1928	0	3	1	6
103	Kipp	Aug. 9, 1928	0	3	1	6
149	Stanmore	Aug. 16, 1928	0	3	2	6
155	Scotfield	Aug. 16, 1928	0	3	1	6
165	Wainwright	Aug. 17, 1928	0	3	1	6
176	Vegreville	Aug. 18, 1928	0	2	0	6
184	Breton	Aug. 23, 1928	0	2	0	6
190	Tofield	Aug. 23, 1928	0	4	0	6
195	Ryley	Aug. 23, 1928	0	3	0	6
199	Holden	Aug. 24, 1928	0	4	0	6
217	Ohaton	Aug. 24, 1928	0	4	0	3
232	Spruce Grove	Aug. 31, 1928	0	3	0	8
246	Spruce Grove	Nov. 26, 1928	0	7	2	19
Totals			0	54	10	113

TABLE II

RESULTS OF ATTEMPTS TO ISOLATE *H. sativum* FROM THE SOIL AND ADJACENT WHEAT STUBBLE COLLECTED AT EDMONTON

No. of sample	Date collected	Isolations of *H. sativum*			
		Soil		Stubble	
		No. of colonies	No. of plates	No. of colonies	No. of pieces stubble
241	Oct. 5, 1928	2	8	0	6
243	Nov. 7, 1928	0	6	6	10
249	Dec. 20, 1928	0	5	3	10
252	Jan. 29, 1929	0	7	1	3
253	Feb. 28, 1929	0	9	1	4
255	Mar. 30, 1929	1	6	1	3
257	April 30, 1929	0	4	0	6
260	May 31, 1929	0	3	0	9
262	June 30, 1929	0	2	0	9
264	July 31, 1929	0	3	3	5
265	Aug. 31, 1929	0	6	3	9
267	Sept. 30, 1929	0	4	0	9
269	Oct. 31, 1929	0	6	0	12
271	Nov. 30, 1929	0	6	0	12
273	Dec. 31, 1929	0	4	2	10
275	Jan. 31, 1930	0	6	4	15
Totals		3	85	24	132

the isolations from the soil in these instances actually came from spores or other parts of the fungus produced on wheat roots in the soil, even though the

precaution of screening out the roots was taken. In the case of summer-fallow where no wheat plants were present to harbor the organism it might be expected that the fungus would be less abundant. The results given in Table III show that it was isolated once from sixteen samples of summer-fallow

TABLE III

RESULTS OF ATTEMPTS TO ISOLATE *H. sativum* FROM SUMMER-FALLOW SOIL AT EDMONTON

No. of sample	Date collected	Isolations of *H. sativum*		No. of sample	Date collected	Isolations of *H. sativum*	
		No. of colonies	No. of plates			No. of colonies	No. of plates
244	Nov. 7, 1928	1	6	261	June 30, 1929	0	3
248	Dec. 20, 1928	0	4	263	July 31, 1929	0	2
250	Dec. 20, 1928	0	5	264	Aug. 31, 1929	0	6
251	Jan. 29, 1929	0	7	266	Sept. 30, 1929	0	4
254	Feb. 28, 1929	0	9	268	Oct. 31, 1929	0	6
256	Mar. 30, 1929	0	6	270	Nov. 30, 1929	0	6
257	April 30, 1929	0	4	272	Dec. 31, 1929	0	4
259	May 31, 1929	0	3	274	Jan. 31, 1930	0	6
				Totals		1	81

soil as compared with thrice from a similar number of samples of soil cropped to wheat. The difference is not sufficient, however, to warrant the conclusion that *H. sativum* is less abundant in the summer-fallow soil. Since the plate method of isolation is probably dependent for its success largely on the presence of spores as has been pointed out by Conn (5), it seemed advisable to ascertain to what extent *H. sativum* sporulates in the soil.

Microscopic Examination of Soils for *H. sativum* Spores

As the spores of *H. sativum* are large and readily distinguished from other fungous spores it was decided to make a direct microscopic examination of soil samples to ascertain if these spores occur under natural conditions in field soils. For this purpose soil samples were collected in a similar manner to that previously described except that in this case a sterile cork borer was used instead of a spatula to take the samples. One set of samples was obtained from the four replicates of a set of plots which had borne three crops of spring wheat in succession. Thirteen distributed samples were taken from each plot, making 52 samples in all. Another set of samples was taken from a large nearby block of fallow soil. Forty-two samples of approximately $\frac{1}{2}$ cu. in. each were taken from different parts of this plot. In the laboratory, each sample was then prepared for examination as follows:— A small thimbleful was measured out in the base of a test tube and transferred to a clean sterile test tube. Then 5 cc. of distilled water was added and the contents shaken for one-half minute. After settling for one minute, 0.07 cc. of the suspension was drawn off with one of the pipettes of a haemacytometer set and transferred to a

clean cover slip. This was then inverted over the counting chamber of the set and immediately examined with the microscope. A careful examination of the samples, 94 in all, failed to reveal spores of *H. sativum* in any of them.

Sporulation of Artificial Cultures of *H. sativum* in Alberta Soils

In order to determine if *H. sativum* could produce spores in the soil it was decided to prepare artificial soil cultures and then to examine them by means of the haemocytometer. Several soils were selected for this purpose, namely, black soil typical of the Edmonton district, brown soil from Brooks, grey soil from Cooking Lake and fine sand from Edmonton. Moisture was added to the water-holding capacity of each, in 200-cc. Erlenmeyer flasks each containing 50 gm. of soil. One series of these was sterilized while another was left unsterilized. Then a tiny bit of a non-sporulating colony of *H. sativum* on water agar was introduced into each and placed on the surface of the soil in a marked position near one side of the flask. After the organism had been allowed time to develop, equal portions of the soil were examined for the presence of spores. Samples were removed from the flask by means of a piece of brass tubing soldered to a steel rod. Two samples of equal surface area, approximately $\frac{1}{8}$ in. in depth, were thus removed about one inch from the piece of agar and mixed in a test tube with 2 cc. of sterile distilled water. After shaking for one minute, each tube was allowed to stand two minutes. Then 0.07 cc. of the suspension was drawn off with a pipette and examined as before by means of the haemacytometer. The results are shown in Table IV.

FIG. 1. *Spores of the same monosporous culture of H. sativum grown on sterilized black soil (left), and on potato dextrose agar (right.)*

It will be seen that the fungus sporulated freely on all samples of sterilized soil but most abundantly on the black soil, though it was not intended to compare the relative sporulation on the different soils, but merely to demonstrate whether the organism could or could not sporulate in the different soils. It is noteworthy that no spores were found in the cultures on any of the unsterilized soils, also that the culture on sterilized soil which produced the fewest spores was contaminated with bacteria. The spores produced on sterilized soil appeared quite normal and germinated vigorously though they were somewhat lighter in color than those produced on potato dextrose agar, Fig. 1. Sporulation on sterilized soil to which organic matter had been added was observed by Christensen (4). Marked differences in the sporulation of different strains of the fungus were also noted by him. In the experiments here reported water only was added to the soil.

TABLE IV

SPORULATION OF *Helminthosporium sativum* ON ALBERTA SOILS

Soil type	Source	Sterilized soil		Unsterilized soil	
		Culture number	Number of spores per cc.	Culture number	Number of spores per cc.
Black	Edmonton	1	8502	21	0
		2	3001	22	0
		3	2834	23	0
		4	1167	24	0
		5	834	25	0
			Av. 3268		Av. 0
Brown	Brooks	6	333	26	0
		7	333	27	0
		8	1667	28	0
		9	2001	29	0
		10	1334	30	0
			Av. 1134		Av. 0
Grey	Cooking Lake	11	1000	31	0
		12	167*	32	0
		13	2167	33	0
		14	667	34	0
		15	333	35	0
			Av. 867		Av. 0
Fine sand	Edmonton	16	834	36	0
		17	333	37	0
		18	4334	38	0
		19	333	39	0
		20	667	40	0
			Av. 1300		Av. 0

**Contaminated with bacteria.*

Another experiment was made using similar methods except that all of the flasks of soil were first sterilized and then the fungus added. Then small quantities of unsterilized soil were introduced into some of the flasks. The amounts added consisted of a trace and one-gram portions. The trace refers to the small amount which adhered to a moist sterilized platinum needle. The results for the Edmonton black soil are shown in Table V. Similar results

TABLE V

EFFECT ON THE SPORULATION OF *H. sativum* OF SMALL ADDITIONS OF UNSTERILIZED SOIL TO STERILIZED SOIL

Soil type	Source	Treatment	Number of spores per cc.
Black	Edmonton	Sterilized	3834
Black	Edmonton	Sterilized	1167
Black	Edmonton	Sterilized + trace unsterilized	0
Black	Edmonton	Sterilized + trace unsterilized	0
Black	Edmonton	Sterilized + 1 gm. unsterilized	0
Black	Edmonton	Sterilized + 1 gm. unsterilized	0

were obtained for the other types referred to in Table IV. As will be noted in Table V sporulation occurred in the sterilized soil alone, but not in the sterilized sample which received either a trace or one-gram portions of unsterilized soil. Evidently the saprophytic micro-organisms of the soil introduced with the unsterilized soil prevented the sporulation of *H. sativum*.

Discussion

While the results obtained indicate that *H. sativum* may occur in field soils which have been cropped to wheat one or more years they do not indicate that it is abundantly present in such soils. It is possible, however, that this fungus may be more common in the soil than the results suggest, if it exists there chiefly in the form of mycelium. As it has been shown that spores of *H. sativum* are very rare if not entirely absent from field soils, it would appear that this fungus if it lives at all in the soil must be largely present in the form of mycelium. The fact that sporulation occurs on sterilized soil but not on unsterilized soil as has been demonstrated, may possibly be explained on the basis of chemical changes in the soil as a result of steam-sterilization, but it would appear that the inhibition of sporulation on unsterilized soil is at least partly attributable to the suppressive action of the normal saprophytic micro-organisms of the soil.

The results may also have a bearing on the survival of the fungus in the soil. If spores are absent, and mycelium only is present, the organism might naturally be expected to be less capable of persisting through periods of adverse conditions, than where both spores and mycelium are present as on most substrata.

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STUDIES ON REACTIONS RELATING TO CARBOHYDRATES AND POLYSACCHARIDES.

XXXVII. THE FORMATION OF DEXTRAN BY *LEUCONOSTOC MESENTERIOIDES*[1]

BY H. L. A. TARR[2] AND HAROLD HIBBERT[3]

Abstract

Four strains of *Leuconostoc mesenterioides* have been studied, and a medium suitable for the production of relatively large amounts of polysaccharide from sucrose has been carefully evolved. All of these organisms brought about polysaccharide formation in nutrient solutions containing sucrose, while two of them showed a slight activity in this respect toward glucose. Melezitose, raffinose, fructose, galactose, lactose, maltose, xylose and glycerol, when employed in the place of sucrose, did not yield any polysaccharide. It is not yet certain if a definite relation exists between the structure of the polysaccharide formed and the sugar utilized, as was found to be the case with levan (8, 9, 10).

Introduction

Pasteur (16) first showed that the slimy fermentation of carbohydrates was due to bacterial action, and assigned to the resulting gummy material the formula $C_{12}H_{20}O_{10}$. Scheibler (12, pp. 461-468) identified a mucilaginous material isolated by him from the juice of sugar beets as being an anhydride of glucose, closely related to starch and dextrin, and therefore named it "dextran." He erroneously believed that the "dextran" slime was formed from the cells of the sugar beet by the action of some enzyme. Jubert (12, p. 462) demonstrated that the slime could be propagated only in beet juice, and that this power of reproduction could be destroyed by heat, or by the addition of phenol, thus giving indirect evidence in support of the idea that the slime formation was caused by a living organism. Mendes (12, p. 462) observed that within the gelatinous masses there were small cells which were able to multiply by fission, thus contributing additional and more conclusive evidence in support of the assumption that the mucilaginous fermentation resulted from the activity of micro-organisms. Durin (5) wholly misinterpreted the phenomenon and decided that the mucilaginous material which he isolated from a vat of German molasses was cellulose, formed from sucrose by the action of the enzymes of the sugar beet. Cienkowski (12, p. 463) recognized the slime produced by this type of fermentation as a true zoogleal formation, and classified the causal organism as *Ascococcus mesenterioides* following the classification of Cohn (12, p. 137). Van Tieghem (12, p. 464) gave the first adequate description of the organism, describing it as similar to the genus *Nostoc* of the green algae, and he gave it the designation which is still extensively used, namely, *Leuconostoc mesenterioides*.

[1] *Manuscript received July 31, 1931.*

Contribution from the laboratories of the Department of Bacteriology and the Department of Industrial and Cellulose Chemistry, McGill University, Montreal, with financial assistance from the National Research Council of Canada and the Canadian Pulp and Paper Association.

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Béchamp (1) proposed the name "viscose" for the viscous products arising from the action of organized ferments on sucrose solutions, and stated that the gum formed was rarely homogeneous. The "viscose" which he examined yielded both glucose and dextrins on hydrolysis, as in the case of starch; in the case of the dextrins from "viscose" the hydrolysis took place more slowly. He agreed with Pasteur (16) that both "viscose" and mannitol were formed during fermentation, but obtained somewhat different proportions of these products. Since neither author describes the organism employed, nor the length of time of fermentation, no definite conclusions can be drawn from their work. It is of interest that Béchamp found that only sucrose was able to undergo the "viscous" fermentation, and that neither invert sugar, glucose, nor fructose could be transformed into "viscose." Däumichen (4) experimented with a mucilaginous material from a sugar factory and found that it resembled Scheibler's dextran in its physical and chemical properties.

Bräutigam (3) isolated an organism from a gelatinous digitalis infusion which he named *Micrococcus gelatinogenes*. This bacterium formed, from the sucrose present in the infusion, a product practically identical with Scheibler's dextran. Both glucose and fructose were found as by-products in the solution. Undoubtedly the *Micrococcus gummosus* isolated and described by Happ (7) was quite closely related to Bräutigam's micrococcus. This bacterium formed a viscous material in solutions containing sucrose or maltose, but Happ made no attempt to study the slime in detail.

Liesenberg and Zopf (13) were the first to isolate strains of *Leuconostoc mesenterioides* in pure culture form, and they succeeded in purifying them by virtue of their resistance to heat. They studied in detail three strains isolated from quite different sources, and their results led them to believe that no marked variation existed among them. In general the properties of these organisms were described by them as follows: When embedded in gelatinous masses they possess a marked resistance to heat, being able to withstand readily a temperature of 75° C. for 15 min. In suitable nutrient solutions which contained either sucrose or glucose, gigantic gelatinous capsules consisting of Scheibler's dextran are formed. The authors found that the gelatinous capsules arose only when the cultures were grown in suitable nutrient solutions containing sucrose or glucose, and that lactose, maltose or dextrin were unsuitable for polysaccharide formation. The strains studied were able to invert sucrose with the formation of fructose and glucose. The addition of relatively large amounts of chlorides (3-5% of calcium chloride, or 1-3% of sodium chloride) accelerated growth, and increased the amount of dextran formed.

Three types of *Leuconostoc* were isolated and described in some detail by Zettnow (19). One was named *Leuconostoc opalanitza*, and a second *Leuconostoc aller*, two variants of this latter organism being described. A detailed study of the cultural and morphological data submitted by Zettnow with reference to these strains appears to indicate that he was dealing merely with growth variants of the same organism, for the differences which he stresses are based merely on the rate and type of growth, as well as with variations in the appear-

ance of colonies of the organisms when grown on sucrose- or glucose-containing nutrient media.

Considerable differences were noted by Liesenberg and Zopf (13) and by Zettnow (19) in the different varieties of *Leuconostoc*. Thus the growth of the strains studied by the former authors was favored by the addition to the medium of fairly large amounts of sodium or calcium chloride, while the growth of the latter author's strains was definitely retarded by similar concentrations of the same salts. While gelatinous capsules corresponding to Scheibler's dextran were formed in media containing either sucrose or glucose by Liesenberg and Zopf's strains, in the case of Zettnow's strains this occurred only with sucrose. Peptone-free solutions, in which the source of nitrogen was asparagine, would not support the development of Zettnow's cultures, while Liesenberg and Zopf noted distinct, though somewhat poor development, of the species studied by them. Again, Zettnow observed that his strains showed definite growth after 24 hr. or somewhat longer, at 8-9° C., while the last-named author's cultures failed to exhibit any visible development at 9-11° C. Morphologically there was, apparently, no visible difference in the various strains studied by the above-named investigators. Probably one of the most significant differences found among the various species studied was that noticed by Liesenberg and Zopf in that the organisms investigated by them were able to withstand a temperature of 75° C. for 15 min. as well as prolonged desiccation, without losing their ability to produce the characteristic "froschlaich" formation in nutrient solutions containing sucrose. Zettnow's strains were exceedingly susceptible to heat and would not withstand drying for even six weeks.

Beijerinck (2) showed that many "dextran-forming" bacteria are merely lactic-acid-producing cocci, which form dextran as a substance of the cell wall. Moreover he showed that these organisms are by no means found occurring only in sucrose and related products, but are also present in natural earth, water, etc. He suggested the name *Lactococcus dextranicus* for this group of bacteria. His studies revealed the fact that only sucrose appears to yield dextran under the influence of these organisms.

Fernbach *et al.* (6) employed Beijerinck's *Lactococcus dextranicus* and noted that it formed a dextrosan only from sucrose, and not from invert sugar, nor from free fructose or glucose, thereby confirming Beijerinck's results.

Seiler (17) examined the slimes produced by several cultures of *Leuconostoc mesenterioides*, and observed that all of them yielded glucose and fructose upon hydrolysis. It is to be observed, in connection with this author's work, that, because of their relative insolubility in water, he did not purify the gums, and that they all contained a fairly large amount of both ash and nitrogen. It is not impossible that some of the unused sugars from the media employed in the preparation of the polysaccharides were mixed with them.

The most recent contribution to our knowledge of the genus *Leuconostoc* has been made by Hucker and Pederson (11), who have classified, mainly by virtue of the total amount of acid produced in solutions of a variety of carbo-

hydrates and polyhydric alcohols, three well-defined species, namely *L. mesenterioides*, *L. dextranicus* and *L. citrovorus*. In the light of this classification it is certain that the organism described by Liesenberg and Zopf (13), and by Zettnow (19), belonged to the species *mesenterioides*, while the type studied by Beijerinck (2), and later by Fernbach *et al.* (6), was evidently of the *dextranicus* species. The main criterion for the differentiation of *L. mesenterioides* and *L. dextranicus* according to Hucker and Pederson (11) is found in the fact that the former ferments the pentoses with the formation of acid, while the latter does not ferment the pentoses.

Lippmann (14) gives probably the most comprehensive summary of the literature dealing with the physical and chemical properties of the dextrans described by various authors. It is quite evident from his account that the various authors who studied these "dextrans" were by no means dealing with homogeneous products in so far as their physical and chemical properties were concerned. In no case has a really detailed study been made of the dextran polysaccharide formed by *L. mesenterioides*. This investigation was therefore undertaken with the intent of studying the conditions surrounding the formation of the dextran polysaccharide by *L. mesenterioides* and at the same time to prepare sufficient pure material for chemical investigations of the product.

Discussion of Results

Four strains of *Leuconostoc mesenterioides* have been studied. A brief survey of their morphological and cultural characteristics, in addition to their ability to form acid from various carbon compounds, indicated that two strains received from Holland were practically the same as the two strains received from Hucker (11), and which had been classified by him as typical strains of this species.

A series of experiments was performed, employing one of the two strains received from Holland, with the view of evolving a simple synthetic medium, and one most favorable for the production of dextran by the strains in question. The results of these experiments indicated that, while a reaction corresponding to pH 8.0 represented the optimum value for polysaccharide formation, this same formation also occurred at values of pH 6.0 to 8.4. A synthetic medium having the following composition appeared to give the best yield of the dextran polysaccharide: peptone, 0.1, sucrose, 10, KCl, 0.1 and $Na_2HPO_4 . 12\ H_2O$, 0.2%. Dextran formation in such a medium apparently reached a maximum 10 days after inoculation.

Subsequent investigation showed that solutions containing sucrose underwent a pronounced "viscous" fermentation with all four strains, while the two cultures received from Holland produced a somewhat similar minor change in solutions containing glucose. When melezitose, raffinose, lactose, maltose, fructose, galactose, xylose or glycerol were employed in place of sucrose, no "viscous" fermentation was observed. When unit volumes of these experimental solutions were treated with five volumes of 95% ethyl alcohol, a pronounced precipitation occurred with all four strains in the nutrient solutions containing sucrose. Slight precipitation also occurred with the two

strains received from Holland in the case of the nutrient solutions containing glucose. Observation indicates that slime formation in the case of glucose is a somewhat transient factor, occurring only when the cultures are very active with respect to the formation of polysaccharide from sucrose.

These results can in general be reconciled with those obtained by Liesenberg and Zopf (13) and by Zettnow (19). The first-named authors worked with strains of *Leuconostoc* which were apparently much more resistant and more active than those studied by the last-named investigator, and they found that marked "dextran" formation took place in nutrient solutions containing either sucrose or glucose. Zettnow however apparently found that his cultures yielded "dextran" only with solutions containing sucrose. It seems probable that only very active cultures of *L. mesenterioides* are capable of forming "dextran" from glucose, although this cannot as yet be stated definitely.

No definite information of a correlation between the structure of "dextran" and the sugar sources which lend themselves to its formation has yet been obtained although the subject is under active investigation. Thus if it be assumed that "dextran" arises from the glucose portion of the sucrose molecule, as has been suggested by Fernbach *et al.* (6), in a manner analogous to the formation of levan from the fructofuranose residue in sucrose (8), then the glucopyranose portion of the melezitose molecule might be expected to give rise to the formation of dextran. However, experimental evidence has shown that this is not the case. Again, there is no apparent reason why these organisms should not be as active with respect to ordinary glucopyranose as they are towards sucrose. At the present time, therefore, it is impossible to state that there is any definite relation between the structure of "dextran" and the sugar source utilized in its synthesis.

Experimental

Culture Media Employed

(1) *Beef infusion broth medium.* Beef infusion broth* with the addition of 0.2% $Na_2HPO_4 \cdot 12H_2O$ and 0.1% KCl. Reaction adjusted to pH 8.0 ± 0.1. (2) *Sucrose beef infusion broth.* Medium (1) with the addition of 10% of sucrose. Reaction adjusted to pH 8.0 ± 0.1. (3) *Sucrose beef infusion gelatine.* Medium (2) with the addition of 15% of gelatine (Bacto). Reaction adjusted to pH 8.0 ± 0.1. (4) *Beef infusion gelatine.* Medium (1) with the addition of 15% of gelatine (Bacto). Reaction adjusted to pH 8.0 ± 0.1. (5) *Sucrose-broth.* Sucrose, 10%; $Na_2HPO_4 \cdot 12\ H_2O$, 0.2%; KCl, 0.5%; peptone, 0.1%; dissolved in distilled water. Reaction adjusted to pH 8.0 ± 0.1. (6) *Sucrose-agar.* Medium (5) with the addition of 1.5% of agar (Bacto). Reaction adjusted to pH 8.0 ± 0.1. (7) *Litmus milk.* Prepared according to the method advocated by the Society of American Bacteriologists (18).

All of the above media were sterilized for 20 min. at 15 lb. steam pressure.

**The beef infusion broth employed was prepared in the following manner: 500 gm. of chopped lean beef was boiled in tap water (one litre) for ¼ hr. The mixture was then strained through cotton wool, and 1% of peptone and 0.5% of NaCl added to the filtrate. The solution was heated for 10 min. in flowing steam, and the pH adjusted to 7.6 by the addition of N/10 NaOH. Subsequently the solution was again heated for 20 min. in flowing steam, filtered through filter paper, and sterilized in an autoclave for 20 min. at 15 lb. steam pressure. The final medium had a reaction corresponding to pH 7.4±0.1.*

Broth for the Determination of "Acid Production" from Various Carbon Compounds

Two solutions were prepared: (a) 1.0% peptone; 0.2% NaCl dissolved in distilled water and the pH adjusted to 6.8±0.1. A 1.6% alcoholic solution (1 cc.) of brom cresol purple, per litre of solution, was added as indicator. (b) A 4% solution of the carbon compound under investigation dissolved in distilled water.

Five cc. quantities of solution (a) were placed in cotton plugged test tubes while solution (b) was placed in an Erlenmeyer flask. All solutions were sterilized for 20 min. at 15 lb. steam pressure. Subsequently they were cooled, and 5-cc. portions of solution (b) added to 5-cc. portions of solution (a), observing aseptic precautions. In this manner 2% solutions of a number of carbon compounds were prepared (see Table I).

Cultures Employed

Through the courtesy of Professor R. O. Herzog, Director of the Kaiser-Wilhelm Institute for Fibre Chemistry, Berlin-Dahlem, Germany, two strains of *Leuconostoc mesenterioides* were obtained from the Technische Hoogeschool Laboratorium voor Mikrobiologie, Delft, Holland. Throughout this investigation these strains have been designated as cultures 1 and 2. In order to ensure pure cultures for experimental work these strains were plated on sucrose-agar, and, after a suitable incubation period at 30° C., representative colonies were transferred to sucrose-broth, and the resulting cultures employed in subsequent experiments.

In addition, several strains of *L. mesenterioides* were obtained from the New York Agricultural Experiment Station through the kindness of Dr. G. J. Hucker. Two of the most active of these, as judged by their ability to form dextran polysaccharide from sucrose broth, were selected for experimental work. These have been designated in this report as cultures 3 and 4: culture 3 being identical with strain 60, and culture 4 with strain 5, as described by Hucker (11).

Throughout this investigation these cultures have been transferred at fairly regular intervals (2-5 days) in either sucrose beef infusion broth, or ordinary sucrose-broth, incubating the cultures at 30° C. In this manner they have been maintained in fairly active condition with reference to the production of "dextran" slime, although, for no apparent reason, the ability to form mucilaginous material from sucrose-broth has varied considerably from time to time, thus making experimental work somewhat difficult.

The only essential variation among the four strains studied appears to lie in their varying ability to produce acid from certain carbon compounds studied, although their morphological and cultural characteristics showed no significant variation. The following general description of the morphological and cultural characteristics is, therefore, applicable to all four of the strains studied.

Cultural Characteristics

Beef infusion broth. Slight turbidity; after approximately three days' incubation a white sediment settles to the bottom of the tube. *Beef infusion*

gelatine stab culture. White, uniform, filiform growth along the line of inoculation; flat, white surface growth, no liquefaction of the gelatine. *Sucrose beef infusion broth.* Rapid development, the medium becomes distinctly opaque and viscous within 24 hr. inoculation, and usually gelatinous within five days of inoculation. After five days' incubation gas bubbles usually appear, and a cartilaginous layer develops at the bottom of the tube. *Sucrose beef infusion gelatine stab.* A profuse, uniform, white, irregular, stalactite-like growth develops along the line of inoculation, and a raised cartilaginous knob forms at the surface. The medium is split by gas formation after about five days. *Sucrose beef infusion gelatine slope culture.* Very abundant, white, raised, irregular, cartilaginous surface growth, which appears to sink into the medium, although the gelatine is not liquefied. *Sucrose-broth.* The development is somewhat slower than in sucrose beef infusion broth. Within 48 hr. of inoculation the medium becomes slightly opalescent and viscous, and, as a rule, becomes distinctly gelatinous within five days of inoculation. *Litmus milk.* Slowly becomes acid with the formation of a very weak clot, and slight, transient reduction of the litmus.

Morphological Characteristics

Gram positive cocci; usually exist as diplococci, although short chains of rarely more than six members are frequently formed. The diameter varies between about 0.5 to 1.0 μ, according to the strain and the medium upon which the cells have been cultivated. Capsules have never been observed, even in very young sucrose-broth cultures, when the Gin method of staining (18) was employed. The slime appears generally to be evenly diffused, and in this respect these strains are, apparently, different from those described by Liesenberg and Zopf (13) and by Zettnow (19), who stated that the organisms studied by them formed huge gelatinous capsules in nutrient media containing sucrose. It is not improbable that the ability of organisms of this genus to form capsules may vary considerably, and that prolonged cultivation under artificial laboratory conditions may involve a transient, or even permanent disappearance of this power.

Production of Acid from Various Carbon Compounds

A study of the ability of the four cultures under investigation to form acid from a variety of carbon compounds was made, chiefly in order to compare the strains received from Holland with those received from Hucker (11) in regard to this power.

Duplicate 10-cc. portions of solutions of the carbon compounds under investigation (see Table I) were inoculated with one 2-mm. loopful of a 48-hour-old sucrose-broth culture of the organism under investigation. Subsequently all the solutions were incubated at 30° C. for 10 days, and the total amount of acid present in each then determined by titrating with $N/10$ NaOH until the original purple color of the indicator returned.

The results of this experiment, which are recorded in Table I, show that all four cultures are very similar in regard to their fermentative power, especially with reference to their ability to form acid from the pentoses (arabinose and

xylose). Thus the two strains received from Holland (cultures 1 and 2) may be considered as typical strains of *Leuconostoc mesenterioides* according to Hucker (11), in regard to their morphology, fermentative power, and cultural characteristics.

TABLE I

AMOUNT OF SODIUM HYDROXIDE REQUIRED TO NEUTRALIZE THE ACID PRODUCED IN 10-CC. PORTIONS OF 2% SOLUTIONS OF THE CARBON COMPOUNDS INDICATED

Carbon compound	No. of cc. of $N/10$ NaOH required to neutralize the acid present in solutions of cultures								Carbon compound	No. of cc. of $N/10$ NaOH required to neutralize the acid present in solutions of cultures							
	1		2		3		4			1		2		3		4	
	(a)*	(b)*	(a)	(b)	(a)	(b)	(a)	(b)		(a)	(b)	(a)	(b)	(a)	(b)	(a)	(b)
Starch	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	Fructose	1.1	1.15	1.6	1.6	0.1	0.15	0.1	0.1
Dextrin	0.1	0.1	0.05	0.05	0.0	0.0	0.0	0.0	Arabinose	0.9	1.0	1.3	1.0	1.4	1.6	1.3	1.3
Inulin	0.0	0.0	0.0	0.0	1.3	1.4	2.3	2.0	Xylose	1.0	0.95	2.5	2.1	1.9	2.2	1.3	1.5
Raffinose	0.25	0.25	0.0	0.0	0.0	0.0	0.0	0.0	Ethylene glycol	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Sucrose	1.0	0.9	1.8	1.8	1.3	1.3	1.5	1.6									
Maltose	0.5	0.4	1.2	1.2	0.8	1.0	0.9	1.0	Glycerol	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Lactose	0.4	0.4	0.0	0.0	0.4	0.4	0.2	0.3	Erythritol	0.1	0.1	0.0	0.0	0.0	0.0	0.0	0.0
Glucose	0.7	0.7	1.55	1.45	0.7	0.8	1.4	1.7	Mannitol	0.0	0.0	1.7	1.9	0.15	0.15	0.2	0.25
Galactose	0.35	0.35	0.5	0.4	0.3	0.4	0.3	0.3	Controls	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

*(*a*) *and* (*b*) *represent duplicate experiments.*

Optimum Conditions for the Production of "Dextran" by Leuconostoc mesenterioides

Owen (15) determined the optimum conditions for the formation of levan by the soil group of bacilli, especially *Bacillus vulgatus*. No author seems to have studied thoroughly the conditions surrounding the production of "dextran" by *L. mesenterioides*. Liesenberg and Zopf (13) noted that the strains of *L. mesenterioides* studied by them apparently exhibited maximum "dextran" formation in an alkaline medium, and it therefore appeared essential to determine first the optimum pH for the formation of polysaccharide by this organism, and to carry out experiments to obtain a simple synthetic medium for the production of dextran for chemical investigations. Culture 1 was employed throughout this series of experiments.

Experiment I. Optimum pH for the Production of Dextran from Sucrose

A solution having the following composition was prepared: sucrose, 10%; KH_2PO_4, 0.5%; NaCl, 0.5%; and peptone, 0.5%; dissolved in distilled water. Quantities (30 cc.) of this solution were brought to various pH values by the addition of $N/1$ NaOH employing the colorimetric method. The approximate amount of $N/1$ NaOH required in each instance to bring the solution to the required pH, together with the indicator employed, is given in Table II.

The solutions were all sterilized separately by filtering through a Berkefeld "W" filter employing suction (500 mm.), and 25 cc. of each was placed in a sterile, cotton-plugged 50-cc. Erlenmeyer flask. The solutions were incubated at 37.5° C. for 48 hr., and at the conclusion of this time showed no visible signs

TABLE II

AMOUNT OF $N/1$ NaOH REQUIRED TO BRING 30 CC. OF THE 10% SUCROSE SOLUTION TO THE pH VALUES INDICATED

Indicator	Brom thymol blue								Phenol red				
Cc. $N/1$ NaOH added (approx.)	0.1	0.3	0.5	0.6	0.7	0.8	0.85	0.9	0.95	1.0	1.05	1.1	1.15
pH of soln. (colorimetric)	6.0	6.2	6.4	6.6	6.8	7.0	7.2	7.4	7.6	7.8	8.0	8.2	8.4

of contamination. Each solution was then inoculated with one 2-mm. loopful of a 48-hour-old sucrose-broth culture of *L. mesenterioides*, and all were incubated at 30° C.

Determinations of the amount of dextran present were made at intervals employing the following technique: 5 cc. of the reacting sucrose solution was withdrawn from the culture flask under aseptic conditions, and placed in a weighed 100-cc. beaker. This solution was made just alkaline to phenolphthalein by the addition of from one to three drops of $N/1$ NaOH, in order to have uniform conditions for precipitation. Ethyl alcohol (95%, 25 cc.) was then added to each beaker, and the dextran permitted to precipitate by allowing the beaker and its contents to stand (covered) at room temperature for 12 hr. The clear, supernatant liquor was carefully decanted from the dextran which adhered firmly to the glass, and the beaker and its contents placed in a vacuum desiccator over $CaCl_2$ and P_2O_5 for 12 hr.; the beaker was then weighed, and the weight of the precipitate calculated by difference. The results of this experiment, recorded in Table III, indicate that dextran can be formed by this organism in both acid and alkaline solution. The optimum reaction, as judged by the greatest amount of polysaccharide formed after ten days' incubation, is at pH 8.0. It is of interest that, while more dextran is formed at pH 6.8 to 7.2 after 2 days' incubation, after ten days' incubation much more of this polysaccharide is obtained from solutions with a pH of about 8.0.

TABLE III

AMOUNT OF DEXTRAN FORMED BY *L. mesenterioides* IN 10% NUTRIENT SUCROSE SOLUTIONS OF VARYING pH

pH of solution	Weight of crude dextran in grams after:			pH of solution	Weight of crude dextran in grams after:		
	2 days	4 days	10 days		2 days	4 days	10 days
6.0	0.0409	0.0436	0.0628	7.4	0.0685	0.0938	0.1410
6.2	0.0857	0.1031	0.1021	7.6	0.0544	0.1166	0.1421
6.4	0.0740	0.0940	0.1065	7.8	0.0586	0.1188	0.1758
6.6	0.0730	0.1071	0.1245	8.0	0.0641	0.1043	0.2173
6.8	0.0831	0.0939	0.1316	8.2	0.0515	0.0913	0.1496
7.0	0.0857	0.0930	0.1391	8.4	0.0513	0.0563	0.1126
7.2	0.0827	0.1052	0.1397				

Experiment II. Optimum Concentration of Peptone for the Formation of Dextran from Sucrose

Portions (30 cc.) of aqueous solutions containing 10% sucrose, 0.2% $Na_2HPO_4 \cdot 12\ H_2O$; 0.5% KCl and varying concentrations (1.0, 0.5, 0.1, 0.05, 0.01, 0.005, 0.001 and 0.0%) of peptone (Bacto) were prepared and the reaction adjusted to pH 8.0 ± 0.1. In all the succeeding experiments the pH of the solutions was brought to the same value and the subsequent treatment was as outlined under experiment 1.

The results (Table IV) show that the optimum concentration of peptone is about 0.05 to 0.1%, and that lower, or higher concentrations, respectively, give lower yields of the polysaccharide.

TABLE IV

EFFECT OF VARYING CONCENTRATIONS OF PEPTONE ON THE AMOUNT OF DEXTRAN FORMED IN TEN PER CENT SUCROSE SOLUTIONS

Percentage of peptone in solution	Weight of crude dextran in grams per 5 cc. of culture solution after:			Percentage of peptone in solution	Weight of crude dextran in grams per 5 cc. of culture solution after:		
	3 days	8 days	30 days		3 days	8 days	30 days
1.0	0.0949	0.0914	0.0857	0.01	0.0092	0.0577	0.0603
0.5	0.0813	0.1067	0.1368	0.005	0.0120	0.0329	0.0547
0.1	0.0793	0.1011	0.1361	0.001	0.0093	0.0323	0.0162
0.05	0.0742	0.1333	0.1414	0.000	0.0112	0.0250	0.0133

Experiment III. Optimum Concentration of Sucrose for the Formation of Dextran from this Sugar (pH 8.0)

Aqueous solutions (60 cc.) were used containing 0.1% peptone, 0.2% $Na_2HPO_4 \cdot 12\ H_2O$, 0.5% KCl, and varying concentrations of sucrose (20, 10, 5, 2 and 1%). The results (Table V) indicate that the higher the concentration of sucrose, up to 20%, the greater the amount of dextran formed. However, the yield of dextran per unit amount of sucrose was greater in the lower than in the higher concentrations. For convenience in experimental work a 10% solution can be employed advantageously, thus avoiding the difficulty occasioned by the presence of sucrose in the "alcohol-precipitated product" employed for chemical investigations.

TABLE V

AMOUNT OF DEXTRAN PRODUCED BY *L. mesenterioides* FROM SOLUTIONS CONTAINING VARYING AMOUNTS OF SUCROSE

Percentage of sucrose in solution	Weight of crude dextran in grams per 5 cc. of culture solution after:				
	3 days	7 days	14 days	21 days	28 days
20	0.0656	0.0724	0.0881	0.1873	0.2141
15	0.0263	0.0333	0.0787	0.1011	0.1882
10	0.0242	0.0320	0.0651	0.0945	0.1453
5	0.0139	0.0254	0.0448	0.0485	0.0942
2	0.0092	0.0101	0.0322	0.0395	0.0397
1	0.0075	0.0099	0.0123	0.0182	0.0236

Experiment IV. Optimum Concentration of $Na_2HPO_4.12H_2O$ for the Formation of Dextran from Sucrose (pH 8.0)

The solutions (60 cc.) used contained 0.1% peptone; 0.5% KCl; 10% sucrose; and varying concentrations of $Na_2HPO_4.12H_2O$ (1.0, 0.5, 0.1, 0.05, 0.01 and 0.00%). The results, recorded in Table VI, show that the optimum concentration of $Na_2HPO_4.12H_2O$ lies between 0.5 and 0.1% at the twenty-first day of incubation, and that at longer time intervals higher concentrations of this salt cause an increase in the amount of polysaccharide formed. Presumably 1% of $Na_2HPO_4.12H_2O$ would give higher yields of dextran on prolonged incubation, but for experimental work, especially for the production of a product low in ash for the chemical investigations, a concentration of 0.2% should prove quite satisfactory.

TABLE VI

EFFECT OF VARYING CONCENTRATIONS OF $Na_2HPO_4.12H_2O$ ON THE AMOUNT OF DEXTRAN FORMED FROM SUCROSE SOLUTIONS BY *L. mesenterioides*

Percentage of $Na_2HPO_4.12H_2O$ in solution	Weight of crude dextran in grams per 5 cc. of culture solution after:				
	3 days	7 days	14 days	21 days	28 days
1.0	0.0608	0.0556	0.0456	0.0983	0.2228
0.5	0.0282	0.0354	0.0883	0.1137	0.1419
0.1	0.0261	0.0249	0.1104	0.1481	0.1276
0.05	0.0162	0.0200	0.0742	0.0868	0.1002
0.01	0.0149	0.0183	0.0608	0.0719	0.0892
0.00	0.0144	0.0181	0.0394	0.0656	0.0810

Experiment V. Optimum Concentration of KCl for the Formation of Dextran from Sucrose

Quantities (60 cc.) of the following solutions containing 0.1% peptone, 10% sucrose, 0.2% $Na_2HPO_4.12H_2O$ and varying concentrations of KCl (1.0, 0.5, 0.1, 0.05, 0.01, and 0.00%) were employed. The results (Table VII) show that, in the early stages of the formation of this polysaccharide, a concentration of 1% of KCl gives the highest yield, while on prolonged incubation the amounts of dextran formed in the solutions containing lower concentrations of this salt become greater, so that finally a concentration of 0.1% KCl appears to be the optimum value. For experimental work a concentration of 0.1% should prove satisfactory, having regard to the isolation of a product readily freed from ash.

The results of the above five experiments, when taken collectively, indicate that a medium having the following composition should prove satisfactory for the experimental production of dextran by *L. mesenterioides:* 10% sucrose, 0.1% peptone, 0.2% $Na_2HPO_4.12\ H_2O$ and 0.1% KCl, the reaction of such a medium being adjusted to pH 8.0 ± 0.1. In view of the fact that good yields of dextran from sucrose could be obtained by the use of the above medium it was not considered necessary to determine the possible increase in the same which might result due to the addition of other inorganic salts

TABLE VII

EFFECT OF VARYING CONCENTRATIONS OF KCl ON THE AMOUNT OF DEXTRAN FORMED BY *L. mesenterioides*

Percentage of KCl in solution	Weight of crude dextran in grams per 5 cc. of culture solution after:				
	3 days	7 days	14 days	21 days	28 days
1.0	0.0862	0.0903	0.1195	0.1473	0.1626
0.5	0.0771	0.0831	0.1401	0.1493	0.1924
0.1	0.0339	0.0383	0.1201	0.1290	0.2195
0.05	0.0332	0.0397	0.1183	0.1246	0.1512
0.01	0.0295	0.0304	0.0512	0.0660	0.0894
0.00	0.0148	0.0237	0.0397	0.0351	0.0315

(magnesium, calcium, etc.), especially since the addition of such salts might make it more difficult to isolate a product practically free from ash for chemical studies.

Experiment VI. Time Required for Maximum Dextran Formation in Sucrose Solutions

The following solutions were prepared: Solution (a). 20% sucrose in distilled water. Solution (b). 0.2% peptone; 0.2% KCl; 0.4% $Na_2HPO_4 . 12H_2O$; dissolved in distilled water, and the reaction adjusted to pH 8.0 ± 0.1.

Two 125-cc. quantities of the above solutions were placed in 300-cc. Florence flasks, sterilized in an autoclave at 15 lb. steam pressure for 20 min., cooled, and mixed, observing aseptic precautions. After inoculation (Culture 1) and incubation, the dextran formed was determined. The results (Table VIII) may be interpreted as showing that dextran formation does not increase after the tenth day of incubation.

TABLE VIII

RELATION BETWEEN TIME AND THE AMOUNT OF DEXTRAN FORMED IN 10% SUCROSE SOLUTIONS

Incubation period in days	Weight of crude dextran in grams per 5 cc. of medium in solution		Incubation period in days	Weight of crude dextran in grams per 5 cc. of medium in solution	
	1	2		1	2
0	0.0030	0.0035	10	0.4110	0.3989
2	0.0519	0.0575	13	0.4105	0.4067
4	0.1611	0.1701	15	0.3888	0.3901
7	0.3607	0.3544	21	0.4131	0.4182
8	0.3789	0.3806	24	0.4010	0.4005
9	0.3765	0.3826	30	0.3981	0.4109

STUDY OF THE SUGARS SUITABLE FOR THE FORMATION OF DEXTRAN BY LEUCONOSTOC MESENTERIOIDES

In view of the fact that levan-forming bacilli were found to exhibit great specificity for the terminal fructofuranose group in sucrose and raffinose (8), experiments were next instituted to ascertain whether or not the strains of

L. mesenterioides under investigation were capable of exerting a selective action only with sugars containing specific sugar residues.

Experiment VII. Polymerizing Action of Four Strains of L. mesenterioides with Reference to the Sugar Involved (pH of solutions = 8 0 ± 0.1)

The solutions used contained 10% of the carbon compound under investigation, together with 0.1% peptone, 0.1% KCl and 0.2% $Na_2HPO_4 . 12\ H_2O$. After sterilizing, employing suction, the solutions were checked by incubating for three days at 30°C. Five separate "sets" representing ten different carbon compounds were prepared. One set was retained as a control, and each of the four remaining sets was inoculated separately from a 48-hour-old sucrose-broth culture of one of the four strains of *L. mesenterioides* being studied. After incubation for 10 days at 30° C., the dextran in each solution was determined as follows:

Ethyl alcohol (95%, 50 cc.) was placed in a weighed 100-cc. beaker, and 10 cc. of the culture fluid added drop by drop. The beakers were covered and the solutions permitted to stand for 12 hr. at room temperature to allow the dextran to settle. The clear, supernatant, alcoholic solution was carefully decanted to avoid disturbing the precipitates, the beakers inverted for 15 min. to remove all the remaining alcoholic solution, and finally the beakers and their contents dried thoroughly by placing in a vacuum desiccator over $CaCl_2$ and P_2O_5 for 24 hr. The results are given in Table IX.

In the case of all four strains the sucrose solutions became distinctly viscous after approximately three days' incubation, and distinctly mucilaginous after ten days' incubation. The solutions containing glucose became slightly viscous in the case of cultures 1 and 2, and apparently remained unchanged with cultures 3 and 4. The weights given in Table IX indicate that dextran is formed in large amounts from sucrose by all four strains, and in small amounts from glucose by cultures 1 and 2. The weights obtained in the case of carbon com-

TABLE IX

DEXTRAN PRODUCTION FROM VARIOUS CARBON COMPOUNDS BY FOUR STRAINS OF *Leuconostoc mesenterioides*

Carbon compound	Weight of dextran in grams per 10 cc. of culture medium in:				
	Culture 1	Culture 2	Culture 3	Culture 4	Controls*
Raffinose	0.0047	0.0056	0.0037	0.0033	0.0035
Melezitose	0.0048	0.0064	0.0053	0.0051	0.0052
Sucrose	0.5051	0.4769	0.2440	0.4549	0.0052
Maltose	0.0043	0.0053	0.0042	0.0033	0.0032
Lactose	0.0039	0.0051	0.0056	0.0041	0.0041
Glucose	0.0257	0.0233	0.0047	0.0039	0.0044
Fructose	0.0056	0.0071	0.0038	0.0027	0.0028
Galactose	0.0061	0.0063	0.0083	0.0060	0.0055
Xylose	0.0057	0.0053	0.0051	0.0045	0.0029
Glycerol	0.0063	0.0049	0.0032	0.0043	0.0041

*The control in each instance represents the residue due to the sugar adhering to the container in the case of the uninoculated controls, which were treated similarly, in other respects, to the inoculated solutions.

pounds other than glucose and sucrose cannot be considered significant when compared with the weights obtained in the control solutions. No formation of a viscous solution took place in solutions containing sugars other than sucrose or glucose.

Preparation of Dextran from Sucrose for Chemical Investigation

The following solutions are prepared: Solution (a). 20% sucrose in distilled water adjusted to pH 7.0 by adding $N/10$ NaOH. Solution (b). 0.2% peptone; 0.4% $Na_2HPO_4.12\ H_2O$ and 0.2% KCl in distilled water, the reaction being adjusted to pH 8.0 ± 0.1.

The above solutions are sterilized separately in one-litre Erlenmeyer flasks in 500-cc. quantities in the autoclave for 20 min. at 15 lb. steam pressure. The resulting solutions are then cooled, mixed carefully under aseptic conditions, inoculated with one 2-mm. loopful of a 48-hour-old sucrose-broth culture of the strain *of L. mesenterioides* under investigation, and incubated for ten days at 30° C. The yield of anhydrous, practically ash-free, pure polysaccharide, calculated on the net weight of sucrose taken varied between 25-35%. The ash content in the purest specimens did not amount to more than 0.05%. The method of purification is to be described in a future paper by Hibbert and coworkers dealing with the chemical structure of dextran, and its application in the study of immunological problems.

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STUDIES ON REACTIONS RELATING TO CARBOHYDRATES AND POLYSACCHARIDES.

XXXVIII. PREPARATION, SEPARATION AND IDENTIFICATION OF THE ISOMERIC BROMOETHYLIDENE GLYCEROLS[1]

By Harold Hibbert[2] and Earl Hallonquist[3]

Abstract

Additional evidence is given regarding the influence of polar radicals, or atoms, on the ease and nature of acetal condensations involving glycerol.

Bromoethylidene glycerol has been prepared as a mixture of the isomeric five- and six-membered ring forms, by the condensation of bromoacetaldehyde with glycerol. This mixture was separated into the two isomeric forms by taking advantage of differences in the physical properties of their benzoates.

The equilibrium ratio of six- to five-membered acetal at room temperature as obtained in the above preparation was found to be 1:15. Under the influence of 1% dry HCl at 25° C. the ratio changed to 1: 8.

By conversion of the acetals into the methyl ethers, and comparison of these with the corresponding products of known constitution obtained by condensation of bromoacetaldehyde with glycerol α- and β-methyl ethers respectively, the identity of each of the acetals was established.

A detailed description is given for the preparation of dibromoparacetaldehyde both in the crystalline form (50% yield) and as a crude syrup (75% yield).

Introduction

Hibbert and Hill (3) in 1923 pointed out for the first time that "the condensation of glycerol (1 mol) with an aldehyde (1 mol) represents an 'intramolecular partition,' and it seems logical to assume that in these reactions two isomers (namely a five- and a six-membered cyclic acetal) will be formed in each case, the relative amounts being dependent on the molecular configuration of the aldehyde employed."

Since that time acetal condensations of glycerol with formaldehyde (1), acetaldehyde (9), trimethylacetaldehyde (11), benzaldehyde (10), *p*-nitrobenzaldehyde (2), cinnamic aldehyde (7), chloral (5) and acetone (4) have been carried out in this laboratory. In all except the latter two cases, both the five- and six-membered ring structures were isolated. In the case of chloral and acetone, only the five-membered acetal could be identified.

In the present investigation bromoethylidene glycerol, formed by condensation of bromoacetaldehyde with glycerol, has been shown to be a mixture of both isomeric forms. The ratio of six-membered to five-membered acetal was found to be 1 : 15.

In a recent paper Hibbert, Morazain and Paquet (5), in discussing the nature of the factors involved in the mechanism of cyclic acetal formation, drew attention to the fact that with a series of alkyl and halogen substituted acetaldehydes, such as the following:

[1] *Manuscript received Sept. 16, 1931.*

Contribution from the Industrial and Cellulose Chemistry Laboratories, McGill University, Montreal, Canada, with financial assistance from the Canadian Pulp and Paper Association.

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Increase in ease of "half-acetal" formation $\longrightarrow$

$(CH_3)_3C—CHO$; $(CH_3)_2CH—CHO$; $CH_3—CH_2—CHO$; $CH_3—CHO$

$CH_2Cl—CHO$; $CHCl_2—CHO$; $CCl_3—CHO$

$\longleftarrow$ Increase in ease of cyclic acetal formation

it is to be expected according to Michael's theory that, as the polar character of the carbonyl (CO) group becomes more negative, the tendency towards addition of water, alcohols and polyhydroxy compounds will increase, the relative stability of the half-acetal formed will increase, and, since the half-acetal is an intermediate product, the relative ease of ring formation will decrease. These authors also suggested that the proportion in which five- and six-membered rings are formed from acetal condensations may be influenced by the polar character of the radicals present. These views were supported by the fact that condensation of trimethylacetaldehyde with glycerol takes place readily and in the absence of a catalyst (11), while with chloral, condensation takes place only under the influence of a strong dehydrating agent such as sulphuric acid or zinc chloride (5). Further, the proportion of six-membered acetal in the case of tertiary amylidene glycerol is relatively high, while it has not been possible to isolate any six-membered cyclic acetal in the case of trichloroethylidene glycerol.

Additional evidence is furnished by the data obtained in the present investigation on bromoethylidene glycerol, as can be seen readily from Table I.

Further work is being carried out in this laboratory with a view to obtaining data on the nature of the products formed from the condensation of isobutyraldehyde, propionaldehyde, dibromoacetaldehyde and bromal with glycerol.

TABLE I

YIELD AND PROPORTION OF ISOMERIC ALKYLIDENE GLYCEROL ACETALS FORMED FROM VARIOUS ALIPHATIC ALDEHYDES

Nature of aldehyde used	Yield of mixed acetals, %	Ratio of isomers at room temperature
$(CH_3)_3C—CHO$	65	1:2
$(CH_3)_2CH—CHO$		
$CH_3—CH_2—CHO$		
$CH_3—CHO$	63	1:4
$CH_2Br—CHO$	35	1:15
$CHBr_2—CHO$		
CCl_3CHO	20	No six-membered isomer formed.

NOTE:—*Data for tertiary amylidene glycerol, ethylidene glycerol, and trichloroethylidene glycerol are taken from references (11), (9), and (5) respectively.*

The ratio indicated in each case is the proportion of six- to five-membered isomer.

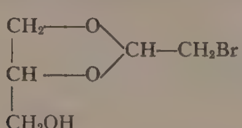
The Bromoethylidene Glycerols

A satisfactory method for the preparation of bromoacetaldehyde, which was required in large quantities for the preparation of crude bromoethylidene glycerol, was de-

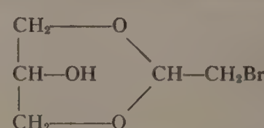
veloped only after considerable experimentation. This method gave constant yields of bromoparaldehyde, either in the form of a crude syrup, which was convenient to use for the preparation of bromoethylidene glycerol, or as pure crystalline dibromoparaldehyde. Bromoethylidene glycerol was prepared according to the method of Hibbert and Hill (3) by condensing bromoparaldehyde with glycerol in the presence of a small amount of sulphuric acid. The mixture of bromoethylidene glycerols so prepared was converted by the action of benzoyl chloride and pyridine into the corresponding benzoates. A crystalline and liquid benzoate resulted and these were separated and purified. The crystalline benzoate, on hydrolysis to the acetal, followed by methylation, gave a methyl ether having properties identical with those of the methyl ether prepared by the condensation of bromoparaldehyde with glycerol β-methyl ether. Similarly the liquid benzoate, on hydrolysis to the acetal, followed by methylation gave a product identical with that obtained by condensation of bromoparaldehyde with glycerol α-methyl ether.

As the bromoethylidene glycerol methyl ether synthesized from glycerol α-methyl ether can only have a five-membered ring, and that from glycerol β-methyl ether a six-membered ring, and since the two compounds differ sufficiently in physical properties, they serve as a means of identifying the five- and six-membered bromoethylidene glycerols respectively, when the latter are converted by methylation to their respective methyl ethers.

The structure of the two isomeric acetals must therefore be as follows:



1: 2-bromoethylidene glycerol.



1: 3-bromoethylidene glycerol.

Table II will serve to compare some physical constants of the acetals and their derivatives.

The slightly higher values for refractive index and density in the case of the 1:2-bromoethylidene glycerol 3-methyl ether obtained by methylation of 1:2-bromoethylidene glycerol, as compared with the values for the 1:2-bromoethylidene glycerol 3-methyl ether prepared synthetically from glycerol α-methyl ether, are presumably due to the fact that the former contained a small amount of 1:3-bromoethylidene glycerol 2-methyl ether. This impurity would raise slightly the values for refractive index, etc. The isolation in the pure state of the five- and six-membered bromoethylidene glycerols is rendered difficult on account of the impossibility of effecting a complete separation of the benzoates. While it is possible to obtain the solid benzoate (6-membered) perfectly pure, it was not found possible to free the liquid benzoate (five-membered) completely from all traces of the solid six-membered isomeric benzoate.

In general the relative values of the physical properties for the respective acetals and methyl ethers follow the trend observed in the case of the ethylidene

TABLE II

PROPERTIES OF THE BROMOETHYLIDENE GLYCEROLS AND DERIVATIVES

Compound	B.p., °C.	M.p., °C.	n_D^{25}	d_4^{25}
1:2-bromoethylidene glycerol	150-151/23 mm.		1.5008	1.6437
1:3-bromoethylidene glycerol	144-145/22 mm.		1.5067	1.6557
1:2-bromoethylidene glycerol 3-benzoate	170/0.6 mm.		1.5452	1.4618
1:3-bromoethylidene glycerol 2-benzoate		109		
1:2-bromoethylidene glycerol 3-methyl ether, prepared from the benzoate	117.5-119/22 mm.		1.4720	1.4694
1:2-bromoethylidene glycerol 3-methyl ether, synthesized from glycerol α-methyl ether	117-119/23 mm.		1.4708	1.4663
1:3-bromoethylidene glycerol 2-methyl ether, prepared from the benzoate	128-129/21 mm.		1.4790	1.4973
1:3-bromoethylidene glycerol 2-methyl ether, synthesized from glycerol β-methyl ether	127-129/21 mm.		1.4792	1.4963

glycerols (9). Thus the 1:3-acetal has a lower boiling point, higher refractive index and higher density than the 1:2-acetal, and the 1:3-methyl ether has a higher boiling point, higher refractive index and higher density than the 1:2-methyl ether, although the differences are not as great as in the cases of most other cyclic acetals.

Experimental

Preparation of Bromoacetaldehyde (*Bromoparaldehyde*)

In spite of numerous attempts it was found impossible to duplicate the method previously outlined by Stepanow *et al.* (12). The following procedure, however, gave satisfactory results.

Pure, freshly distilled paraldehyde (105 gm.) was placed in a three-necked flask fitted with mercury seal stirrer, dropping funnel and calcium chloride drying tube. Bromine (300 gm.), dried over concentrated sulphuric acid and distilled, was added drop by drop through the separatory funnel, the contents of the flask being stirred vigorously and maintained at −15° C. by immersion in a cooling bath. The addition of the bromine took about six hours. The mixture was then stirred for about five hours at −15° C. At the end of this time 150 gm. of sodium acetate, finely divided, and in the form of an aqueous sludge, was very slowly added, the temperature being kept below 0° C. The mixture was stirred for a few minutes and then allowed to stand at −10° C. for 10 hr. Enough water was added to dissolve the excess of sodium salts and the mixture was extracted four times with ether. The ether solution was placed in a large flask and stirred with an electrically driven stirrer, the temperature being maintained at −10° C. by immersion in the cooling bath. Sodium hydroxide (5%) was slowly added till the mixture was neutral to litmus.

The ether solution, which previously had been slightly brown in color, was now perfectly colorless. The ether layer was washed free from alkali with a small amount of water, further washed with concentrated sodium bisulphite solution, then again with water and finally dried over anhydrous sodium sulphate. The ether was distilled off under reduced pressure (water-pump). A very light straw-colored syrup (180 gm.) remained, representing a yield of 75% of crude product. This syrup, on standing overnight, crystallized. By filtering with suction, and recrystallizing the solid from warm alcohol, 120 gm. of pure dibromoparaldehyde (m.p. 57° C.) was obtained. Yield, 50%. This is the product prepared with so much difficulty by Stepanow *et al.* (12). It was found, however, to be more convenient to use the crude syrup in the preparation of bromoethylidene glycerol, and better yields were obtained than with the pure crystalline dibromoparaldehyde.

Preparation of Crude Bromoethylidene Glycerol

Crude syrupy bromoparaldehyde (170 gm.) and 128 gm. of pure glycerol were placed in a round-bottomed flask equipped with a mechanical stirrer. Sulphuric acid (40%, 15 drops) was added, and the contents of the flask slowly heated on the steam bath with good stirring. No appreciable reaction seemed to take place until the temperature reached 80° C. The reaction mixture was stirred at 80-90° C. for 20 hr., a slight amount of discoloration taking place. The mixture was extracted with ether, the ethereal solution neutralized with dilute sodium hydroxide solution, washed with a small amount of sodium bisulphite solution, then with water, and dried over anhydrous sodium sulphate. The ether was distilled off under reduced pressure and the remaining liquid fractionated under reduced pressure, yielding 111 gm. of substance, b.p. 145-155° C./22 mm. On refractionation 95 gm. of a pure bromoethylidene glycerol, b.p. 142-144° C./17 mm., was obtained (bath temp. 175-180° C.). Yield, 35% on basis of glycerol used.

Preparation of Benzoates of Bromoethylidene Glycerol

Dry HCl gas was bubbled into 251 gm. of bromoethylidene glycerol (prepared as above) until 2 gm. had been absorbed. The mixture was allowed to stand for 48 hr. at 25° C. It was then dissolved in 100 gm. of dry pyridine, and this solution added slowly, and with cooling, to a solution of 178 gm. of benzoyl chloride in 120 gm. of pyridine. The resulting reaction mixture was allowed to stand overnight at 40° C. It was then dissolved in ether, the ether solution washed several times with 1% aqueous sulphuric acid, then with dilute sodium bicarbonate solution, and finally with water. The ether extract was dried over anhydrous sodium sulphate, and the ether recovered by distillation. The residual, very viscous oil amounted to 336 gm., representing a yield of 87% of crude product. This viscous oil exhibited no tendency to crystallize when cooled to a low temperature, even on standing for several weeks, but at room temperature it began to crystallize and soon became a thick paste. All attempts to isolate the crystalline part of this paste by fractional precipitation from solvents failed. In all accessible solvents the difference in solubility between the solid and liquid benzoate was so slight, or

else the proportion of solid to oil was so small, that no separation could be made. Ligroin, alcohol, benzene, ether, toluene, ethyl acetate, chloroform, acetone, carbon tetrachloride and suitable combinations of these solvents failed to give any separation.

By spreading the paste on a porous plate, the oil was absorbed, and a solid left almost free from oil. This solid was then scraped off, dissolved in hot ligroin or ligroin-benzene mixture, and on cooling crystallized beautifully in rosettes of rhombic plates. Yield, 23 gm., m.p. 109° C. Analysis: calcd. for $C_{12}H_{13}O_4Br$, Br, 26.60; found, 26.54%. Further experiments showed this substance to be 1:3-bromoethylidene glycerol 2-benzoate.

The oil absorbed by the porous plate was extracted with ether in a Soxhlet extraction apparatus, the ether recovered by distillation and the oil thus obtained fractionated under reduced pressure, giving 175 gm. (45.6% yield) boiling at 170° C./0.6 mm. (bath temp. 205° C.); n_D^{25}, 1.5452; d_4^{25}, 1.4618. Analysis: Calcd. for $C_{12}H_{13}O_4Br$, Br, 26.60; found, 26.46%. Further experiments showed this to be 1:2-bromoethylidene glycerol 3-benzoate. Total amount of pure benzoates obtained, 175 gm.+23 gm.=198 gm. Total yield of pure benzoates, 51%.

The proportion of solid benzoate to liquid benzoate is thus 23:175 or 1:8. This proportion is obtained only when the crude bromoethylidene glycerol is treated with HCl prior to formation of benzoates. An experiment carried out as above, but without a pre-treatment of the bromoethylidene glycerol with HCl, gave a proportion of solid to liquid of only 1:15. Thus HCl appears to cause a shift towards the six-membered isomer in the mutual interconversion of five- and six-membered acetals which takes place under the influence of acid (6). The proportions given above are only approximate and vary slightly in different experiments.

Hydrolysis of 1:2-Bromoethylidene Glycerol 3-Benzoate

Fifty-six gm. of 1:2-bromoethylidene glycerol 3-benzoate was treated with 12 gm. of sodium hydroxide in 120 cc. of water together with 10 cc. of 95% alcohol. The mixture was heated to 35° C. and shaken in an automatic shaker for 12 hr., at the end of which time a clear homogeneous solution resulted. Potassium carbonate (100 gm.) was added and the mixture extracted with ether. The precipitated sodium benzoate was filtered off and both the aqueous layer and the sodium benzoate precipitate were extracted several times with ether. The ether solution was dried over anhydrous potassium carbonate and the ether distilled off. The residue was fractionated under reduced pressure, giving 16.9 gm. of 1:2-bromoethylidene glycerol, b.p. 150-151° C./23 mm. Yield, 46%; n_D^{25}, 1.5008; d_4^{25}, 1.6437. Analysis: Calcd. for $C_5H_9O_3Br$, Br, 40.60; found, 40.85%.

Hydrolysis of 1:3-Bromoethylidene Glycerol 2-Benzoate

Thirty-five gm. of 1:3-bromoethylidene glycerol 2-benzoate was treated with 7.5 gm. of sodium hydroxide in 75 cc. of water together with 6 cc. of 95% alcohol, in exactly the same manner as in the preceding experiment. Twenty-four hours were required for complete solution. The six-membered

benzoate seemed to be more resistant to hydrolysis than its five-membered isomer, and also some decomposition and replacement of bromine was noted. After extraction, distillation yielded 3 gm. of 1:3-bromoethylidene glycerol. B.p. 144-145° C./22 mm.; n_D^{25}, 1.5067; d_4^{25}, 1.6557. Analysis: Calcd. for $C_5H_9O_3Br$, Br, 40.60; found 40.47%.

Methylation of 1:2 Bromoethylidene Glycerol

Dimethyl sulphate (25 gm.) and a solution of 15 gm. of sodium hydroxide in 21 cc. of water were allowed to drop from separate tap-funnels into 25 gm. of the well-stirred acetal during a period of two hours, the temperature being kept at 35-40° C. and a slightly alkaline reaction being maintained throughout. A solution of 3 gm. of sodium hydroxide dissolved in 25 cc. water was then added and the reaction mixture heated at 70° C. for one hour. The cooled reaction product was then extracted with chloroform, dried over anhydrous sodium sulphate and distilled; 10.6 gm. of 1:2-bromoethylidene glycerol 3-methyl ether was obtained. B.p. 117.5-119° C./22 mm.; n_D^{25}, 1.4720; d_4^{25}, 1.4694. Analysis: Calcd. for $C_6H_{11}O_3Br$, Br, 37.92; found, 37.95%.

Methylation of 1:3-Bromoethylidene Glycerol

The reaction was carried out in the same manner as with the 1:2-bromoethylidene glycerol, except that the following quantities were used: 1:3-bromoethylidene glycerol, 3.5 gm.; sodium hydroxide in 3 cc. of water, 2.1 gm.; dimethyl sulphate, 3.5 gm. On extracting and distilling 1.1 gm. of 1:3-bromoethylidene glycerol 2-methyl ether was obtained. B.p. 128-129° C./21 mm.; n_D^{25}, 1.4790; d_4^{25}, 1.4973. Analysis: Calcd. for $C_6H_{11}O_3Br$, Br, 37.92; found, 38.15%.

Synthesis of 1:2-Bromoethylidene Glycerol 3-Methyl Ether

Pure glycerol α-methyl ether (82 gm.; b.p. 110° C./13 mm.; n_D^{17}, 1.4462; d_4^{17}, 1.1198), 95 gm. of bromoparaldehyde (crude syrup), and 8 drops of 40% sulphuric acid were heated at 80-90° C. in a round-bottomed flask with vigorous stirring for 24 hr. as in the preparation of bromoethylidene glycerol. No charring occurred, the mixture at the end of this time having a light straw-colored appearance. It was extracted with ether, the ether solution neutralized with dilute sodium hydroxide solution, washed with a solution of sodium bisulphite, then with water and dried over anhydrous sodium sulphate. The ether was distilled off and the residual liquid fractionated under reduced pressure. Sixty-two gm. of 1:2-bromoethylidene glycerol 3-methyl ether (b.p., 117-119° C./23 mm.; n_D^{25}, 1.4708; d_4^{25}, 1.4663) was obtained. Yield, 38%. Analysis: Calcd. for $C_6H_{11}O_3Br$, Br, 37.92; found, 37.80%.

Synthesis of 1:3-Bromoethylidene Glycerol 2-Methyl Ether

Glycerol β-methyl ether (5.7 gm.); b.p. 123° C./13 mm.; d_4^{17}, 1.1306; n_D^{17}, 1.4505 and prepared from 1:3 benzylidene glycerol 2-methyl ether (8), was treated with 6.6 gm. of bromoparaldehyde as in the preceding experiment. There was obtained in the final distillation 3.5 gm. of 1:3-bromoethylidene glycerol 2-methyl ether; b.p., 127-129° C./21 mm.; n_D^{25}, 1.4792; d_4^{25}, 1.4963. Analysis: Calcd. for $C_6H_{11}O_3Br$, Br, 37.92; found, 38.21%.

It is the authors' intention to investigate the action of alkalies on each of the bromoethylidene glycerols.

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TWO-COMPONENT SYSTEMS INVOLVING COMPOUND FORMATION[1]

By J. Russell[2] and O. Maass[3]

Abstract

The two-component systems ethyl ether-hydrogen chloride and methyl alcohol-hydrogen chloride have been examined in the gaseous state, and from the pressure-volume-temperature relationship of the binary mixture evidence is adduced of the existence of compound formation. The heats of reaction appear to be constant in the temperature range investigated, and are 5400 calories for the ether hydrochloride, and 9200 calories for the alcohol hydrochloride. The pressure-volume-temperature data for ethyl ether, methyl alcohol and ethyl alcohol are given over the temperature range 50-200° C., and over the pressure range below one atmosphere.

When two gases are mixed the pressure-volume-temperature relationship of the mixture is in general different from that calculated on the basis of Dalton's Law. In every case new factors enter which cause aberration from the ideal gas laws.

One such factor is the attraction between the two different species of molecules. When equal volumes of two gases, each at a pressure of one atmosphere, are mixed, a pressure change takes place which is generally very small, but which is dependent on the above-mentioned factor. Occasionally a marked decrease in pressure occurs, far greater than would be accounted for by the attraction indicated in van der Waals' equation, and which is due to a partial combination with the formation of complex molecules. A large number of systems of the former type where no combination takes place have been studied by Sivertz and Maass (5) and by B. P. Sutherland and Maass (6), and the results will be published shortly. In this paper a number of systems of the latter type in which the pressure changes seem to indicate compound formation are described.

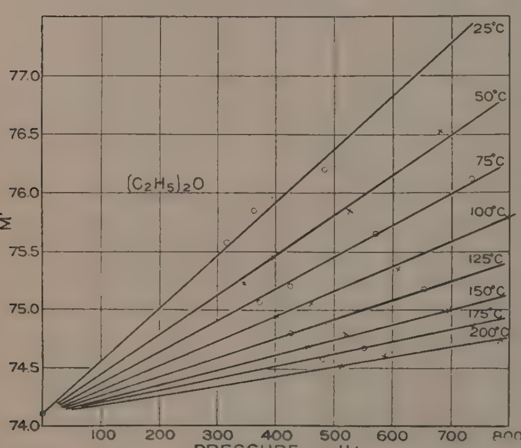

FIG. 1. *Apparent molecular weight of ethyl ether at various pressures and temperatures.*

Alcohols and ethers form molecular complexes (oxonium compounds) with hydrogen chloride when mixed together in the liquid state. Maass and Morrison (3) examined the system methyl ether-hydrogen

[1] *Manuscript received July 16, 1931.*
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chloride in the gaseous state, and showed that an oxonium compound was formed in this case also. This was confirmed independently by J. Shidei (4). The systems described below are ethyl ether-hydrogen chloride and methyl alcohol-hydrogen chloride. Since it was necessary for purposes of calculation to know the pressure-volume-temperature data of the single components these were first determined. Incidentally, these data for ethyl alcohol were also determined.

Preparation of Materials

The hydrogen chloride was prepared by dropping concentrated c.p. sulphuric acid upon concentrated aqueous c.p. hydrochloric acid, the evolved gas being passed through a wash bottle containing concentrated sulphuric acid, then through a tube of phosphorus pentoxide and finally condensed in a tube immersed in a bath of ether cooled to the condensation point of hydrogen chloride by means of liquid air. The liquid obtained was then distilled twice through tubes containing phosphorus pentoxide, the final condensation taking place in a container attached to the density apparatus. It was kept in the liquid state and admitted to the apparatus as desired.

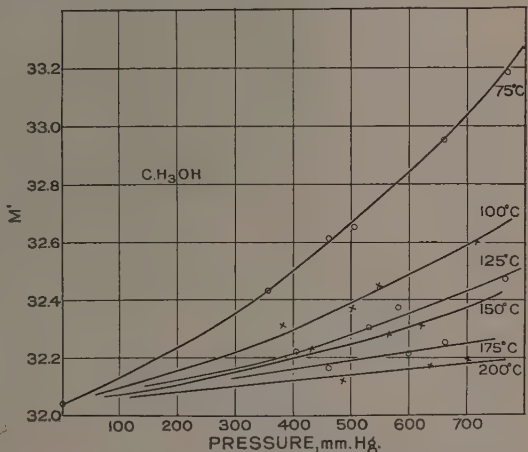

FIG. 2. *Apparent molecular weight of methyl alcohol at various pressures and temperatures.*

The ethyl ether was prepared in the usual manner. Starting with a so-called pure product, it was thoroughly washed with water to remove alcohol and given a preliminary drying by means of calcium chloride. Repeated distillations over sodium gave a product in which no impurity could be detected.

The methyl alcohol was prepared from a c.p. product. It was first treated with a few crystals of iodine to remove the acetone, distilled first over potassium hydroxide and then three times over metallic calcium. The final product showed no sign of impurity.

The ethyl alcohol was treated first with potassium hydroxide to resinify the aldehydes, distilled from the potassium hydroxide and then repeatedly distilled over metallic calcium. (The presence of the aldehyde had not been suspected at the start, so the first preparations were impure.) A careful determination of the density of this final product was made and the result, $d_4^{25} = 0.78508$, was found to be in almost exact agreement with the Bureau of Standards value, 0.78506. In point of fact the actual experimental work reveals any appreciable amount of impurity which may be present in any of

the substances, since the extrapolation of the isotherms to zero pressure should give the value $M' = M$, where M is the theoretical molecular weight. This was always found to be so except in the case of the first alcohol samples, mentioned above, which were of course discarded.

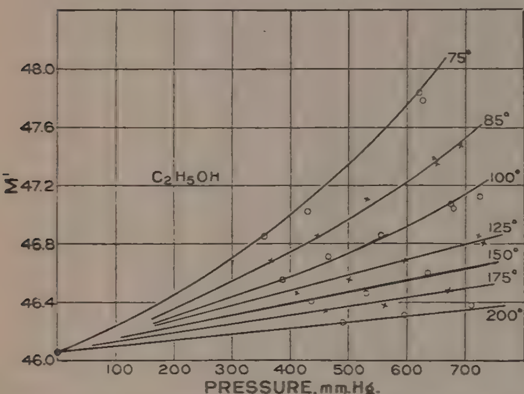

FIG. 3. *Apparent molecular weight of ethyl alcohol at various pressures and temperatures.*

Apparatus and Method

The experimental method was practically identical with that devised by Maass and Mennie (2) for the investigation of substances which are liquid at room temperatures, and for measuring the pressure-volume-temperature relations of a two-component system.

Experimental Results

In Tables I, II and III are given the data obtained for ethyl ether, methyl alcohol and ethyl alcohol. They are tabulated in terms of M', the apparent molecular weight calculated from the quantities observed assuming the ideal gas law to hold.

TABLE I

DATA OBTAINED WITH ETHYL ETHER

Temp., °C.	Mass	Pressure, mm. Hg	Volume, cc.	M'	Temp., °C.	Mass	Pressure, mm. Hg	Volume, cc.	M'
25.0	2.0548	482.5	1039.0	76.203	125.3	2.0523	652.3	1040.1	75.168
25.0	1.5340	361.9	1039.0	75.847	125.3	1.5321	488.9	1040.1	74.870
25.0	1.3355	316.2	1039.0	75.575	125.3	1.3340	426.1	1040.1	74.798
50.1	2.0542	525.2	1039.3	75.862	151.0	2.0519	695.7	1040.3	74.998
50.1	1.5334	394.2	1039.3	75.447	151.0	1.5317	520.8	1040.3	74.786
50.1	2.6796	679.1	1039.3	76.532	151.0	1.3336	454.1	1040.3	74.678
50.1	1.3351	344.2	1039.3	75.232					
					175.5	2.0513	736.6	1040.6	74.882
75.5	2.6787	736.0	1039.5	76.123	175.5	1.5313	551.4	1040.6	74.674
75.5	1.3348	371.9	1039.5	75.070	175.5	1.3333	480.8	1040.6	74.568
75.5	2.0536	567.9	1039.5	75.637					
75.5	1.5329	426.3	1039.5	75.208	201.2	2.0508	779.9	1040.9	74.738
					201.2	1.5309	583.2	1040.9	74.608
100.8	2.0530	611.1	1039.8	75.348	201.2	1.3330	508.4	1040.9	74.520
100.8	1.5325	458.0	1039.8	75.045					
100.8	2.6780	792.6	1039.8	75.780					
100.8	1.3343	399.4	1039.8	74.928					

The discussion of the values for the one-component systems is left in abeyance at present due to the improved method of Cooper and Maass (1) and their modification of the equation proposed by Maass and Mennie (2) which is to

TABLE II

Data obtained with methyl alcohol

Temp., °C.	Mass	Pressure, mm. Hg	Volume, cc.	M'	Temp., °C.	Mass	Pressure, mm. Hg	Volume, cc.	M'
75.5	1.0373	658.4	1039.5	32.953	151.0	0.7890	621.0	1040.3	32.307
75.5	0.7897	505.8	1039.5	32.656	151.0	0.7184	565.9	1040.3	32.281
75.5	0.7189	461.1	1039.5	32.610	151.0	0.5478	432.2	1040.3	32.229
75.5	1.2210	769.6	1039.5	33.184					
75.5	0.5482	353.5	1039.5	32.436	175.5	0.7888	657.7	1040.6	32.249
					175.5	0.7182	599.6	1040.6	32.209
100.8	1.0371	713.4	1039.8	32.605	175.5	0.5476	457.9	1040.6	32.156
100.8	0.7894	545.6	1039.8	32.451					
100.8	0.7188	498.0	1039.8	32.372	201.2	0.7886	696.3	1040.9	32.189
100.8	0.5481	380.4	1039.8	32.315	201.2	0.7180	634.3	1040.9	32.172
					201.2	0.5475	484.4	1040.9	32.124
125.3	1.0368	762.8	1040.1	32.474					
125.3	0.7892	582.5	1040.1	32.369					
125.3	0.7185	531.5	1040.1	32.298					
125.3	0.5479	406.3	1040.1	32.217					

TABLE III

Data obtained with ethyl alcohol

Temp., °C.	Mass	Pressure, mm. Hg.	Volume, cc.	M'	Temp., °C.	Mass	Pressure, mm. Hg.	Volume, cc.	M'
75.5	0.9677	430.4	1039.5	47.027	125.3	0.9671	496.4	1040.1	46.545
75.5	0.7972	355.9	1039.5	46.851	125.3	0.7968	409.8	1040.1	46.453
75.5	1.4192	620.5	1039.5	47.838	125.3	1.1618	594.7	1040.1	46.673
75.5	1.4265	624.5	1039.5	47.776	125.3	1.4165	722.4	1040.1	46.845
					125.3	1.4256	727.9	1040.1	46.792
85.6	0.9675	444.4	1039.6	46.851					
85.6	0.7971	367.5	1039.6	46.676	151.0	0.9668	529.2	1040.3	46.454
85.6	1.1623	530.8	1039.6	47.122	151.0	0.7965	436.4	1040.3	46.411
85.6	1.4171	643.7	1039.6	47.376	151.0	1.1615	633.8	1040.3	46.599
85.6	1.5269	692.0	1039.6	47.483					
85.6	1.4262	648.1	1039.6	47.350	175.5	0.9666	560.4	1040.6	46.380
					175.5	0.7963	462.1	1040.6	46.336
100.8	0.9674	464.5	1039.8	46.710	175.5	1.1611	671.7	1040.6	46.480
100.8	0.7970	384.0	1039.8	46.550					
100.8	1.1621	556.2	1039.8	46.861	201.2	0.9663	593.0	1040.9	46.314
100.8	1.4169	675.1	1039.8	47.072	201.2	0.7961	489.1	1040.9	46.261
100.8	1.5267	726.6	1039.8	47.123	201.2	1.1608	711.4	1040.9	46.375
100.8	1.4260	679.9	1039.8	47.039					

be published in the near future. In Fig. 1, 2 and 3, M' is plotted against the pressure. In the cases of methyl and ethyl alcohols the curvature of the isotherms at the lower temperatures indicates a certain amount of association.

The data for the two-component systems are given in Tables IV and V. The pressure calculated is obtained from the data given above, and the previously determined data for hydrogen chloride. The method of calculation is given in the paper by Maass

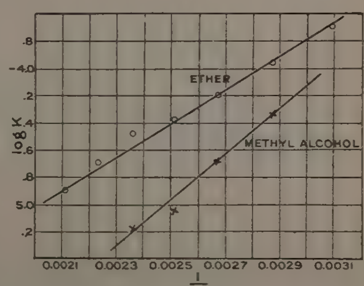

Fig. 4. *Relation between inverse of the absolute temperature and logarithms of the mass law constants for the ether and alcohol systems.*

and Mennie (2). The last column gives the difference in pressure between calculated and observed pressures of the mixtures.

TABLE IV

DATA FOR THE SYSTEM ETHYL ETHER-HYDROGEN CHLORIDE

Temp., °C.	P_{Ether}, mm. Hg	P_{HCl}, mm. Hg	$P_{calc.}$, mm. Hg	$P_{obs.}$, mm. Hg	Difference, mm. Hg
Ether, 1.0019 gm; HCl, 0.5047 gm.					
50.1	258.3	267.1	525.4	512.9	−12.5
75.5	279.0	288.0	567.0	558.8	− 8.2
100.8	299.5	308.8	608.3	602.3	− 6.0
125.3	319.4	328.8	648.2	643.9	− 4.3
151.0	340.2	350.0	690.2	686.3	− 3.9
175.5	360.0	370.0	730.0	727.3	− 2.7
201.2	380.7	391.0	771.7	769.8	− 1.9
Ether, 1.1680 gm.; HCl, 0.5920 gm.					
50.1	300.5	313.3	613.8	596.4	−17.4
75.5	325.3	337.8	663.1	651.6	−11.5
100.8	348.6	362.1	710.7	703.7	− 7.0
125.3	371.9	385.8	757.7	752.0	− 5.7

TABLE V

DATA FOR THE SYSTEM METHYL ALCOHOL-HYDROGEN CHLORIDE

Temp., °C.	P_{CH_3OH}, mm. Hg	P_{HCl}, mm. Hg	$P_{calc.}$, mm. Hg	$P_{obs.}$, mm. Hg	Difference, mm. Hg
75.5	317.2	315.9	633.1	628.5	−4.6
100.8	431.1	338.8	679.9	677.5	−2.4
125.3	363.6	360.9	724.5	723.3	−1.2
151.0	387.4	384.0	771.4	770.4	−1.0

NOTE: *Methyl alcohol, 0.4928 gm.; HCl. 0.5539 gm.*

Discussion of the Results

The deviations tabulated above, *i.e.*, the differences between the observed and calculated pressure, are of a greater magnitude than the changes due to the mixture effect. If this latter is neglected the mass law constant can be calculated on the assumption that the observed changes are due to the existence of the equilibria

$$HCl + (C_2H_5)_2O \rightleftharpoons (C_2H_5)_2O.HCl \text{ and}$$

$$C_2H_5OH + HCl \rightleftharpoons C_2H_5OH.HCl.$$

In Table VI are given the logarithms of the mass law constants for the ether and alcohol systems at various temperatures, and in Fig. 4 these are plotted against the inverse of the absolute temperature.

TABLE VI

LOGARITHMS OF THE MASS LAW CONSTANTS FOR THE ETHER- AND ETHYL ALCOHOL-HYDROGEN CHLORIDE SYSTEMS AT VARIOUS TEMPERATURES

T	$\frac{1}{T}$	log K_{Ether}		log K_{CH_3OH}
		1	2	1
50.1	0.00309	−3.699	−3.682	—
75.5	0.00287	−3.966	−3.950	−4.34
100.8	0.00267	−4.171	−4.239	−4.68
125.3	0.00251	−4.376	−4.388	−5.04
151.0	0.00236	−4.475	—	−5.17
175.5	0.00223	−4.687	—	—
201.2	0.00211	−4.890	—	—

Since the slopes of the curves in Fig. 4 are linear the heats of reaction appear to be constant in the temperature range investigated, and are 5400 calories for the ether hydrochloride, and 9200 calories for the alcohol hydrochloride.

In Table VI it was possible to calculate two values for the mass law constant in the case of the ether from two sets of data in which the amounts of material used were different. The agreement for each pair of values is such as to give additional evidence that the interpretation of the data is based on an assumption which is probably correct.

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THE VAPOR DENSITY OF HYDROGEN SULPHIDE[1]

By R. H. Wright[2] and O. Maass[3]

Abstract

An apparatus for the measurement of the vapor density of condensable gases at pressures greater than atmospheric and at various temperatures is described. The vapor density of redistilled hydrogen sulphide was measured at 47°, 25°, 0°, −20°, and −35° C., and up to 4 atm. pressure. The interpolated value for the vapor density of hydrogen sulphide under standard conditions was found to be 1.537. This value agrees with the values given by Leduc (1.538) and Baume and Perrot (1.539), but the difference may be attributed in part to the neglect of the earlier experimenters to allow for deviations from the gas laws in correcting their values to standard pressure. The results are expressed as apparent molecular weights, and the deviations from the theoretical value shown to be due to the action of molecular attraction.

Introduction

This paper deals with a series of measurements on the vapor density of hydrogen sulphide, undertaken in connection with an investigation of aqueous solutions of the gas and as an extension of the work of this laboratory on deviations from the ideal gas laws. The only precision measurements of this quantity to be found in the literature are those of Leduc (3) and Baume and Perrot (1) which refer only to standard temperature and pressure. Hitherto no comprehensive vapor density measurements above atmospheric pressure have been carried out in this laboratory in checking the modified van der Waals' equation proposed by Maass and Mennie (4), and found to be very satisfactory at the lower pressures. In this work the range has been extended to four atmospheres and the theory found capable of accounting for the facts as observed.

Purification of Hydrogen Sulphide

Compressed liquefied hydrogen sulphide from a cylinder was dried in a trap at −50° C. and purified by low temperature fractional distillation *in vacuo*. The perfect condensability of the product in liquid air and the ability to cross-check vapor density measurements with different batches testified to the purity of the final product.

Measurement of Vapor Density

The procedure was essentially that of Maass and Russell (5) which has also been found to be satisfactory by succeeding workers in this laboratory.

The apparatus is shown in Fig. 1. A calibrated volume, Q, of Pyrex glass, and fitted with a pressure stopcock, P, was connected through a second pressure stopcock, N, with the low pressure portion of the system which included the condensation bulbs, M M, low pressure manometer, F, storage bulb, at G, and pumps. On the high pressure side was the manometer, S, four metres

[1] *Manuscript received August 10, 1931.*
Contribution from the Physical Chemistry Laboratory, McGill University, Montreal, Canada. Constructed from a thesis presented in partial fulfilment of the requirements for the degree of Doctor of Philosophy in the Department of Chemistry, McGill University.

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high. The volume, Q, was of 586.7-cc. capacity at 25° C. and was shaped to fit inside a large Dewar flask used as a low temperature thermostat. This was filled with acetone, stirred by a stream of air and hand regulated to within 0.05° C. For temperatures above 0° C. an automatically regulated water bath was used. Temperatures were read on mercury and alcohol thermometers graduated in tenths of a degree and furnished with calibration certificates by the manufacturer.

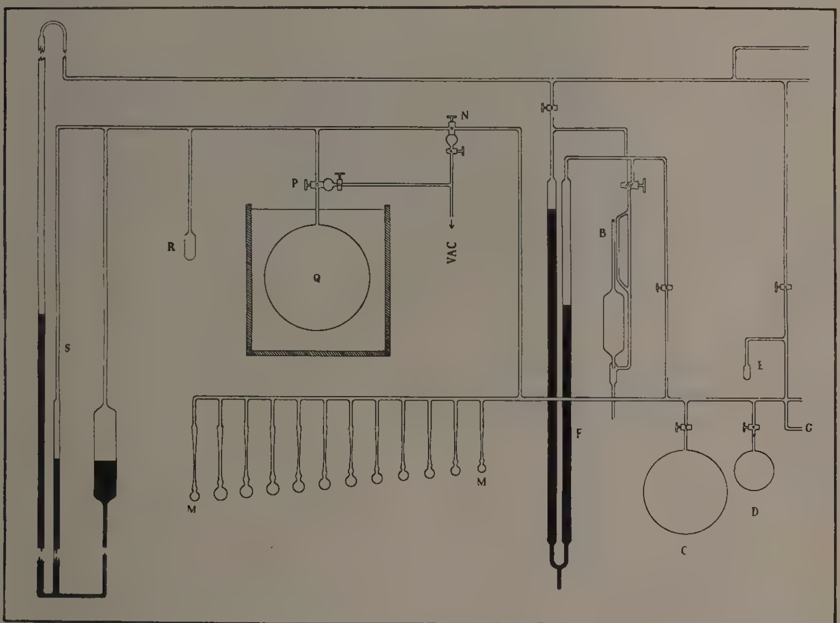

FIG. 1. *Diagram of apparatus.*

In making a determination of vapor density, the whole apparatus was thoroughly evacuated and the gas admitted from G into the entire high and low pressure systems (including C and D) until the pressure was something under an atmosphere. The hydrogen sulphide was condensed in R with liquid air and any residual gas pumped out. Pressure tap N was closed and R allowed to warm up, on which a considerable pressure was developed depending on the original pressure in F. This pressure was measured to the nearest 0.5 mm. on S and tap P closed, whereupon the gas in the tubing was removed. One of the bulbs, M, was then placed in liquid air and the gas in Q condensed into it as completely as possible. Any residual pressure was read on F.

The bulb M was sealed off, allowed to come to room temperature, and weighed to 0.1 mg. It was cooled again in liquid air and the neck broken off (care being taken to lose none of the glass) and the hydrogen sulphide allowed to escape. The empty bulb was dried in a vacuum desiccator and

re-weighed. It was finally filled with distilled water and weighed a third time to determine the volume of air contained in it during the second weighing. The difference between the first and second weighings gave the weight of hydrogen sulphide originally in Q except for the small amount left in the apparatus in sealing off the bulb. Knowing the volume of the tubing and the pressure of the residual gas, this correction was readily found from the ideal gas law, hence the weight of gas initially occupying the known volume, Q, under fixed conditions of temperature and pressure was found. The calculation of vapor density and apparent molecular weight, and the corrections applied to the various measurements were the same as those described by Maass and Mennie (4).

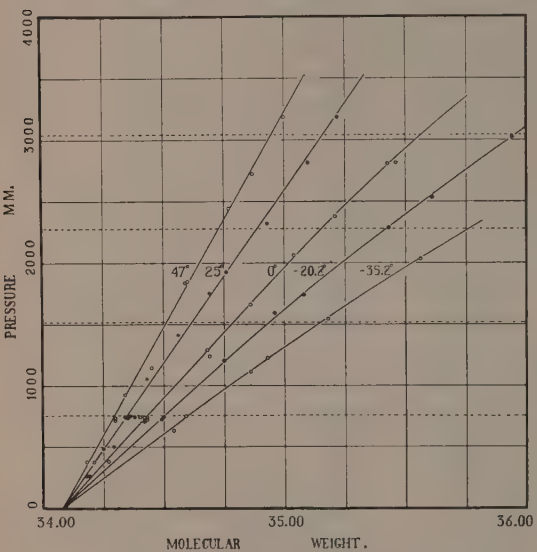

FIG. 2. *Apparent molecular weights of hydrogen sulphide at various temperatures and pressures.*

Experimental Results

The experimental results are tabulated for each temperature in Table I, wherein are shown the pressures (in mm. of mercury at 0° C.) and corresponding apparent molecular weights. These molecular weights are plotted in Fig. 2.

The vapor densities of hydrogen sulphide under stand-

TABLE I
EXPERIMENTAL RESULTS

Temp., °C.	Pressure, mm. Hg.	M'	Temp., °C.	Pressure, mm. Hg.	M'	Temp., ° C.	Pressure, mm. Hg.	M'
47.0	3187	35.00	25.4	750.5	34.36	0.0	740.9	34.42
	2724	34.87	25.1	743.2	34.38		726.2	34.42
	2442	34.77	25.4	743.3	34.34		725.3	34.43
	1850	34.60	25.1	740.3	34.35		382.0	34.27
	1843	34.59	25.4	492.8	34.25	−20.2	3021	35.94
	1148	34.45		502.2	34.29		2537	35.61
	922	34.34	25.5	263.7	34.19		2289	35.43
47.1	735.8	34.30		260.4	34.18		1742	35.08
	718.6	34.30	0.1	2815	35.46		1594	34.96
	375.1	34.21		2814	35.43		1204	34.75
	378.8	34.18		2380	35.21		749.6	34.50
25.0	3185	35.22	0.0	2067	35.04		727.4	34.49
	2819	35.10		1661	34.86	−35.2	2035	35.56
	2321	34.93		1292	34.68		1545	35.18
	1928	34.76		1240	34.69		1228	34.93
	1752	34.69		721.8	34.42		1116	34.86
	1418	34.56		744.7	34.40		751.2	34.59
	1056	34.43		743.1	34.43		631.7	34.54

ard conditions given by Leduc (3) and Baume and Perrot (1) are 1 538 and 1 539 gm. per litre respectively, while the interpolated value for this work is 1.537. This agreement is within the range of experimental error, but the difference may be attributed in part to the neglect of the earlier experimenters to allow for deviations from the gas laws in correcting their values to standard pressure.

Discussion of the Results

The modification of van der Waals' equation proposed by Maass and Mennie (4) is based on the interdependence of van der Waals' b and the molecular mean free path. This, in turn, is known to be influenced by temperature, even at constant volume, due to the action of molecular attraction, as shown by Sutherland. In order, therefore, to obtain an expression giving the variation of b with temperature, the mean free path used in deriving the equation is expressed in terms of the Sutherland viscosity-temperature relation, which may be assumed to define the variation with temperature of the mean free path, corrected for the influence of molecular attraction. The resulting equation takes the form,

$$\left(P+\frac{a}{V^2}\right)\left(V-B\left(1+\frac{c}{T}\right)\right)=RT, \tag{1}$$

or

$$PV^2-RTV+a-RTB\left(1+\frac{c}{T}\right)=0. \tag{2}$$

In the subsequent discussion, the following conventions in symbols will be adhered to: P = pressure in atmospheres, V = volume of 1 gm.-mol. in litres, $R=0.08206$ = gas constant in litre-atmospheres, T = absolute temperature, c = Sutherland's constant, and a and B = constants.

In applying the above equation it is first necessary to evaluate a and B. Knowing the diameter of the molecules and Sutherland's constant for the gas, B can be obtained from the relation,

$$B=\frac{8\sqrt{2}\,\pi r^3 N}{1+\frac{c}{273.1}}, \tag{3}$$

where r = radius of the molecule, N = Avogadro's Number $=6.061\times10^{23}$.

Rankine and Smith (6) give for the viscosity of hydrogen sulphide at 0° C. the value $k=116.6\times10^{-6}$ poise, from which r is obtained by substitution in the following formula, due to Jeans (2),

$$k=\frac{0.499m\overline{C}}{2\pi d^2}, \tag{4}$$

where k = coefficient of viscosity, m = mass of one molecule, $\overline{C}$ = average velocity $=\sqrt{\frac{8}{3\pi}}$ (rt. mean sq. velocity), and d = diameter of one molecule. On making this substitution, $r=2.362\times10^{-8}$ cm.

Returning to Equation 3 and substituting the value for r, then, $B=0.1283$. Writing

$$b=B\left(1+\frac{c}{T}\right), \tag{5}$$

and substituting the Rankine and Smith value for c, *viz.*, 331, the series of

values of b given in Table II are obtained.

TABLE II

VALUES OF b OBTAINED BY SUBSTITUTING RANKINE AND SMITH'S VALUE FOR C IN EQUATION 5

Temp., °C.	47.0	25.0	0.0	−20.2	−35.2
$B(1+\frac{c}{T})$	0.2610	0.2708	0.2839	0.2963	0.3069

In order to evaluate a, the value of b for 0° C. and 1 atm. pressure, and corresponding molecular weight and volume data from the experimental results are substituted in Equation 2, which gives $a = 11.46$.

Equations 1 and 2 can now be applied to the data obtained over the whole range of temperature and pressure. By substituting Equation 5 in Equation 1, at constant temperature, it reduces to $PV^2 - RTV + a - RTb = 0$. Maass and Mennie showed that on substituting $V = \frac{M}{M_0} - \frac{RT}{P}$, the resulting equation could be written,

$$\frac{M}{M_0} = 1 + \left(\frac{a - RTb}{R^2T^2}\right)P + 2\left(\frac{a - RTb}{R^2T^2}\right)^2 P^2 + \text{etc.} \tag{6}$$

Since the series converges rapidly, especially at reasonably high temperatures, they assumed that the apparent molecular weight varies linearly with the pressure, $\frac{M}{M_0} = 1 + AP$, where $A = \frac{a - RTb}{R^2T^2}$.

As will be seen from Fig. 2, this is actually the case with hydrogen sulphide up to quite high pressures, and at temperatures above 0° C. At 0° C., however, a slight curvature can be detected which becomes more pronounced as the temperature falls. It is therefore necessary to take account of the higher powers of P in Equation 6 if high pressures or low temperatures are encountered.

In Table III the molecular weights at 1, 2, 3 and 4 atmospheres pressure, taken from the curves of Fig. 2, are compared with molecular weights cal-

TABLE III

COMPARISON OF CALCULATED AND EXPERIMENTALLY DETERMINED MOLECULAR WEIGHTS OF HYDROGEN SULPHIDE

Temp., °C.	Pressure in atmospheres							
	1		2		3		4	
	Molecular weight							
	Calc.	Obs.	Calc.	Obs.	Calc.	Obs.	Calc.	Obs.
47.0	34.30	34.30	34.54	34.52	34.78	34.73	35.02	34.94
25.0	34.35	34.35	34.64	34.62	34.94	34.89	35.26	35.16
0.0	34.43	34.43	34.79	34.79	35.18	35.15	35.58	35.56
−20.2	34.49	34.50	34.96	34.94	35.42	35.42	35.93	35.95
−35.2	34.57	34.60	35.12	35.15	35.67	35.76	—	—

culated from Equation 6 making use of the first two terms of the series in P. The agreement is everywhere within the range of experimental error, with the possible exception of $-35.2°$ C. and 3 atm. pressure. The "observed" molecular weight at this point is taken from the extrapolated curve of Fig. 2, and as this is very close to the condensation point, the point may actually be in the region of supersaturation. Unfortunately the literature contains no data pertaining to the phase equilibria near this temperature.

This is the first time this curvature of the molecular weight versus pressure isotherms has been compared with the curvature predicted by the theory, and the closeness of the agreement is entirely satisfactory.

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A MODIFIED FLOW METHOD FOR MEASURING THE VELOCITIES OF GAS REACTIONS[1]

By E. W. R. Steacie[2] and H. A. Reeve[3]

Abstract

A modified flow method has been devised for measuring the velocities of gas reactions, which enables observations to be made by pressure readings rather than by tedious analytical methods. In principle the method consists of passing the reactants through a flow-meter, then through a heated reaction chamber, and finally through a second flow-meter. The ratio of the two flow-meter readings, corrected for the change in the viscosities of the gases, gives a direct measure of the extent to which the reaction has progressed. As a test of the method the thermal decomposition of ammonia on the surface of silica has been investigated.

Introduction

Two main methods have been used for the kinetic investigation of gas reactions. In one of these the pressure change is measured as the reaction proceeds in a bulb at approximately constant volume. This method possesses the advantages of simplicity and directness of measurement. It has, however, two main disadvantages. In the first place the method is not suitable for the investigation of very rapid reactions. In addition, the analysis of the products of the reaction is difficult, on account of the very small quantity of material formed.

In the flow method, the reactants are passed through a hot tube, and the rate of the reaction is deduced from the rate of flow and the analysis of the products of the reaction. The main advantage of this method is the large amounts of the products which are available for analysis. Its main disadvantage is the large number of analyses which are necessary in order to determine the rate of the reaction.

The present communication deals with an attempt to devise an apparatus which will combine the advantages of the two methods discussed above. As a test of the method, the thermal decomposition of gaseous ammonia has been investigated.

Apparatus

In principle the method consists of passing the gaseous reactants through a flow-meter, then through a hot tube in which partial reaction occurs, and finally through a second flow-meter. If in the reaction a change in the total number of molecules occurs, then the amount of gas passing the second flow-meter will differ from the amount passing the first.

If the viscosity of the mixture of products is the same as that of the mixture of reactants, the difference between the two flow-meter readings will be a direct measure of the extent to which the reaction has progressed during the time that the reactants were in the heated tube. The method thus allows the

[1] *Manuscript received May 30, 1931.*
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actual observations to be made by means of pressure readings on the flow-meters, and at the same time provides ample material for analysis.

In general, however, the viscosity of the mixture of gases formed will not be the same as that of the reactants. The difference between the flow-meter readings will therefore not be a direct measure of the extent to which the reaction has progressed. The amount of reaction may however be calculated either from a knowledge of the viscosities of the gases concerned, or from the ratio of the two flow-meter readings when the conditions are such that the reaction goes to completion.

The apparatus is illustrated in Fig. 1. The reaction chamber was a transparent quartz tube, *D*, on which was wound two layers of 16 gauge nichrome wire, separated from the quartz tube and from each other by asbestos paper. On the outside of the windings a further layer of asbestos paper, about one inch thick, was wound for heat-insulating purposes. The windings could be used either in series or in parallel, the current being controlled by suitable rheostats.

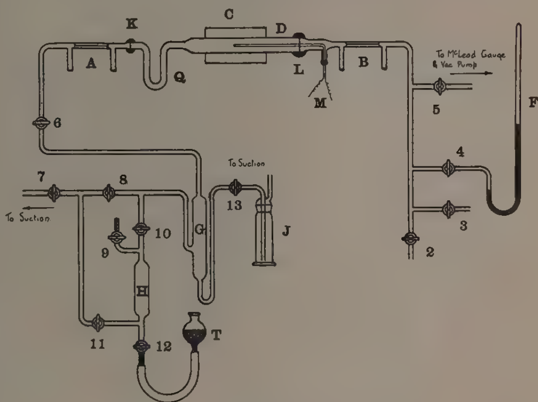

FIG. 1. *Diagram of apparatus.*

At each end of *D* the flow-meters, *A* and *B*, were connected by deKhotinsky seals, *L* and *K*. To prevent softening of the wax, these seals were cooled by a stream of water. The volume of gas (uncorrected for viscosity changes) passing through *A* and *B* was given by pressure readings on the attached manometers, which were filled with paraffin oil. The method of calibration of these flow-meters will be described later. The temperature of the reaction chamber was given by the chromel-alumel thermocouple, *M*, in conjunction with a Leeds and Northrup potentiometer indicator. The temperature could be controlled and read with an accuracy of about 2° C. The thermocouple was enclosed in a thin quartz tube to prevent any possible catalytic action of the chromel or alumel. The leads were taken out through a side tube and deKhotinsky seal, as shown.

The hot gases leaving the reaction chamber were cooled in their passage through the quartz U-tube, *Q*, which was immersed in cold water.

The reactant (ammonia) was introduced from a cylinder, and its rate of flow controlled by tap 3. Its pressure was given by the mercury manometer, *F*. Tap 5 led to the pumping system, which consisted of a Langmuir pump backed up by an oil pump together with a McLeod gauge.

The trap, *G*, was intended for the condensation of liquid products of reaction. These could then be transferred to *J* by suction and withdrawn for analysis.

Gaseous products were drawn into *H*, and withdrawn for analysis through tap 9. With ammonia, when samples were not being kept for analysis, the unchanged reactant and the products of the reaction were finally passed through tap 7, and then bubbled through water and dilute hydrochloric acid.

Calibration

To determine the quantity of gas passing through the apparatus, corresponding to any given readings of the gauges, the following method of calibration was used. Two Drexel gas washing bottles containing water were connected in series to the apparatus at tap 7. Ammonia was then passed through the apparatus at a constant rate, as indicated by the gauge readings, for a measured time interval. The bottles were weighed before and after, and the volume of ammonia passing through the apparatus per minute was calculated on the assumption that ammonia obeys the ideal gas law at room temperature and atmospheric pressure. The relation between rate of flow and gauge reading was linear within the experimental error.

Since the two flow-meters were not of exactly the same dimensions, the readings on them for the same rate of gas flow differed slightly. They were therefore calibrated one against the other. The relation between the two gauges was also practically linear.

Experimental

The furnace was first brought to the desired temperature. Ammonia was then admitted through tap 3, and its rate of flow adjusted by means of the valve on the ammonia cylinder to give some constant reading of gauge *B*. If the temperature of the furnace were high enough, some of the ammonia decomposed on passing through *C*. The reading on gauge *A* was therefore higher than that of gauge *B*, since the decomposition of ammonia is attended by a volume increase, and, in addition, the viscosity of the mixture of products is higher than that of ammonia. Readings were taken when gauge *A* indicated a constant value, showing that equilibrium conditions had been established in *C*. A series of observations was made in this way with various rates of flow. Experiments were carried out over a temperature range from 900° to 1300° C.

To determine the effect of surface on the velocity of decomposition, another series of runs was made with the reaction chamber packed with small quartz rods.

With the packed tube, it was possible to obtain complete decomposition of the ammonia at measurable rates of flow at the higher temperatures employed. It was found that when the ratio of the two gauge readings, expressed as litres of ammonia per minute, was 2.69, analysis of the products showed that complete decomposition had occurred. This ratio therefore represented the maximum possible decomposition, and further increasing the temperature had no effect.

Since the decomposition of ammonia proceeds according to the equation

$$2\ NH_3 \longrightarrow N_2 + 3\ H_2,$$

we would expect that the volume of gas passing the second flow-meter would be double the volume passing the first when complete decomposition occurs. We would therefore expect a maximum ratio of the two flow-meter readings of 2.0.

The higher ratio actually obtained, *viz.*, 2.69, is presumably due to the fact that the viscosity of the hydrogen-nitrogen mixture formed is not the same as that of ammonia.

The gauge reading of a capillary flow-meter can be represented as follows (1):

$$h = \frac{K_1 \, \eta \, R \, l}{d^4} + \frac{K_2 \, D \, R^2}{d^4},$$

where h = gauge reading, K_1 and K_2 = constants, η = viscosity, R = rate of flow, l = length of capillary, d = diameter of capillary and D = density of the gas. The second term in this equation makes allowance for end effects, and can be neglected since long capillaries were used. For the same flow-meter, we may therefore write

$$h \propto \eta R$$

or in other words, the gauge reading will be proportional to the rate of flow and to the viscosity of the gas.

It follows, therefore, that the ratio of gauge readings, corresponding to complete decomposition, should be $\frac{2.0\ \eta_1}{\eta_2}$, where η_1 is the viscosity of a 3 to 1 hydrogen-nitrogen mixture and η_2 that of ammonia.

These viscosities have not been determined experimentally at very high temperatures. According to Kleint (4) the viscosity of a 3 to 1 hydrogen-nitrogen mixture is 0.000134 at 0° C. At this temperature the viscosity of ammonia is 0.000093 (5). The ratio of the two viscosities is 1.44. Hence, if the temperature coefficients of the two viscosities were identical, the limiting ratio of the two flow-meter readings would be 2.88. This is sufficiently close to the observed value of 2.69, since the assumption of identical viscosity-temperature coefficients is certain to be in error.

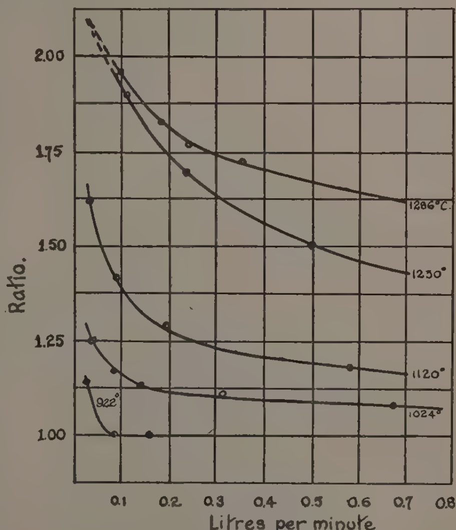

FIG. 2. *Flow-meter ratio—rate of flow curve with empty reaction chamber.*

Since the reaction is heterogeneous, the rate was much slower when the reaction chamber was not packed with silica rods. In consequence, a sufficiently high temperature could not be reached for complete decomposition. The maximum ratio obtainable in this case was about 2.0. Analysis of the products of the reaction, however, showed that some ammonia

was still undecomposed. The amount of ammonia agreed with the assumption that the reaction had only gone $\frac{2.0}{2.69}$ to completion.

The ratio 2.69 may therefore be taken as denoting complete decomposition, and the amount of decomposition under any conditions can be calculated from the ratio of the two flow-meter readings.

Calculations and Experimental Results

The complete data for a typical run at 1120° C. with an unpacked tube are given in Table I.

TABLE I

DATA FOR A TYPICAL RUN AT 1120° C. WITH AN UNPACKED TUBE

Gauge *B*		Gauge *A*		Ratio $\frac{A}{B}$	% Reacted	Time sec.
Mm. of oil	Litres per min. (expressed as NH_3)	Mm. of oil	Litres per min. (expressed as NH_3)			
10.5	0.045	17	0.071	1.57	33.8	25.6
20	0.100	30	0.137	1.37	21.9	12.2
40	0.188	54	0.240	1.27	16.0	6.7
60	0.270	77	0.332	1.23	13.6	4.7
80	0.355	100	0.425	1.19	11.2	3.7

The first and third columns represent the actual experimental observations on the two flow-meters. The second and fourth columns represent these readings converted to give the volume of gas passing each flow-meter in unit time. These volumes are expressed as though the gas in each case were pure ammonia. The ratio of the two flow-meter readings is given in the fifth column. This serves as a measure of the extent to which the reaction has progressed during the time that the reactant was in the hot part of the tube. The values of this ratio at various temperatures and rates of gas flow are given in Fig. 2 for the empty tube and in Fig. 3 for the tube packed with quartz rods. The per cent reaction is given in the sixth column. This is calculated on the assumption that a ratio of 2.69 corresponds to complete decomposition, and that the per cent reaction and the ratio of the flow-meter readings vary in a linear manner.

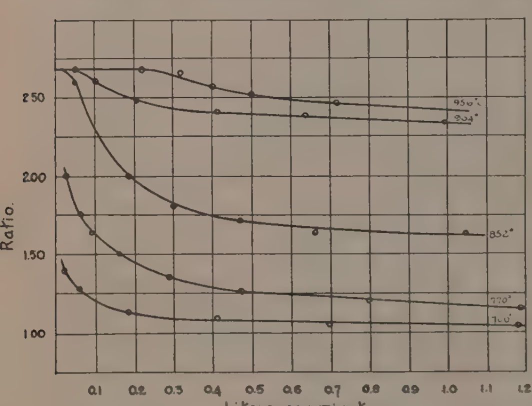

FIG. 3. *Flow-meter ratio—rate of flow curve with reaction chamber packed with quartz rods.*

In order to obtain typical reaction-velocity curves giving the amount reacted

corresponding to various times, it is necessary to know the time during which any amount of gas is in the furnace. This may be determined from a knowledge of the rate of gas flow and the volume of the furnace. Some uncertainty arises, however, on account of the fact that the amount of gas, and hence its rate of flow, changes continuously throughout the furnace as reaction occurs. The actual relationship between the rate of flow and the distance along the furnace is complicated. For our purpose, however, it will be sufficiently accurate to assume that the rate of flow through the furnace is the mean of that corresponding to the initial volume of the gas as it enters the furnace and the final volume as it leaves (these volumes, of course, being calculated at the temperature of the reaction chamber). The times calculated in this way are given in column 7 of Table I. Fig. 4 gives typical per cent reaction-time curves calculated from the data of Fig. 3.

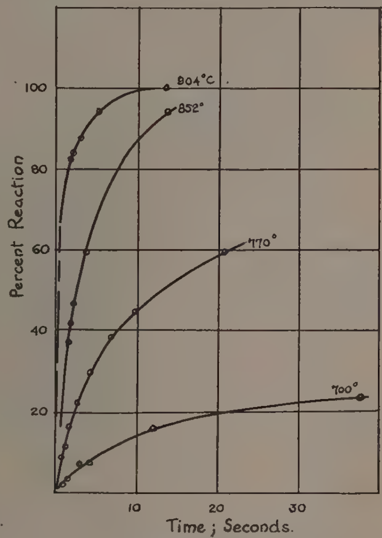

FIG. 4. *Typical per cent reaction—time curves calculated from the data of Fig. 2.*

Discussion

Since the present communication is merely a description of the method, the complete results obtained with ammonia will not be given here except in so far as they have a bearing on the reliability of the method of measurement under discussion.

The results obtained are in agreement with what would be expected from theoretical considerations, *i.e.*, the curves given in Fig. 2 and 3 approach the theoretical straight lines for an infinitely fast and an infinitely slow reaction as the rate of flow and the temperature are varied.

The actual results with ammonia confirm the reliability of the method since they are in general agreement with those of Bodenstein and Kranendieck (2, p. 99) and of Hinshelwood and Burk (3). The reaction has no definite integral order, either the first or the second, since the adsorption of hydrogen formed during the reaction retards the rate. The retardation found was in general agreement with the results of Hinshelwood and Burk.

As far as the method is concerned it may be said that considerably greater accuracy would be needed to make it of general applicability. This could probably be attained by the use of more sensitive manometers of the differential type. The complicated nature of the hydrodynamics of the system is also somewhat of a drawback. Such complications, however, are common to all flow methods.

There are, however, two specific cases in which the present method should prove of value. First, in the investigation of the decomposition of organic compounds, particularly the gaseous hydrocarbons. In such reactions direct readings of the volume change accompanying the reaction could be obtained, together with the possibility of collecting large amounts of liquid products which might be formed in very small amount. Secondly, it should prove useful in giving a continuous direct reading method of determining the yield in industrial catalytic reactions.

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ECONOMY OF TIME IN LABORATORY DISTILLATION[1]

By D. F. Stedman[2]

Abstract

The mathematics of fractional distillation of ideal mixtures has been condensed, so that the most economical "reflux ratio" for any such mixture may be decided at once.

Particular use is made of the "critical reflux ratio" for any mixture, above which even an infinite column cannot obtain complete separation; and the relation of this critical value to the most economical value for any particular case is given.

Some of the conclusions with respect to the infinite column were tested by means of a mixture of methyl and ethyl alcohols using a particularly efficient column. It was found that the vapor produced in the still contained slightly more than the theoretical amount of methyl alcohol, and the magnitude of such error is illustrated from previous work on glycerine solutions.

The results are given in the form of a graph of the "critical reflux ratio" for the case where the most volatile constituent boils at 100° C., and the difference between the boiling points varies from 0.25° C. to 32° C., the concentration of the most volatile constituent also being included from 0.001 to 1.0.

A table of correction factors is also given, showing the factor by which the "critical reflux ratio" should be varied to produce the greatest economy of time for any particular case.

This paper is intended primarily as a condensation of the available data on fractionation of ideal mixtures, in such a form that it may be of direct application to laboratory work. Many chemists may almost be said to spend their lives using distillation as a casual tool for the separation of every conceivable mixture, in much the same manner as others more fortunately situated use filtration.

Distillation is however a much more complex operation than filtration, and in work which has been under way for some time a very serious need has been felt for condensed data which would give some idea of what might be reasonably expected from any particular distillation.

A very large number of distillations are made even on entirely unknown synthetic mixtures, where the only guiding data may be a rough idea of the boiling points of the constituents into which the mixture separates from a preliminary distillation. In such cases where no vapor data can possibly be available, and in others which do not warrant the preliminary study necessary to obtain such data, the use of mathematical calculations will obviously give only the roughest idea of what to expect, but even in these cases some suggestions may be obtained as a guide.

Much data may be obtained from the literature with reference to bubbling columns, but the laboratory column is more generally a packed one, or one closely approximating to it mathematically, and in any case no data have been previously presented in a sufficiently condensed form to be readily available at a moment's notice.

This paper therefore develops the equations covering the operation of a

[1] *Manuscript received July 14, 1931.*
Contribution from the National Research Laboratories, Ottawa.
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packed column separating an ideal mixture, and presents the results in a form which may be applied directly.

The following terms, similar to those used by Walker, Lewis and McAdams (5, p. 597) may be defined:—

x = mol fraction of lighter constituent, a, in original mixture.
x_p = mol fraction of a in product obtained.
y = mol fraction of a in equilibrium vapor produced from mixture. The still is assumed to provide the column with this vapor.
T and $T+\Delta t$ = the respective boiling points of constituents.
P = mols of liquid taken off as product per minute.
V = mols of vapor entering bottom of column per minute.
α = volatility ratio.

By definition

$$\alpha = \frac{y}{1-y} \cdot \frac{1-x}{x}, \tag{1}$$

$$\therefore \quad y = x\left(\frac{\alpha}{1+x(\alpha-1)}\right). \tag{2}$$

Throughout a packed column the rate of doing work is directly proportional to the amount that the vapor diverges from equilibrium with the liquid with which it is in contact.

This fact is put into a basic equation by Thormann (4, p. 89), but although his method may be worked out plate by plate quite readily, the dependence of the result on the reflux ratio is not mathematically self-contained and must therefore be worked out.

Let N = the maximum number of condensations and re-evaporations which may be obtained from the column, when all vapor is condensed and returned as reflux. Under these conditions the liquid is of the same composition as the vapor at all points, and is therefore "one theoretical plate" out of equilibrium with the vapor, and the rate of doing work is at a maximum. Also let the fraction of this amount of work which may be obtained in practice be k.

When taking off P mols of product per minute the number of theoretical plates to which the column and still is equivalent is therefore,

$$kN+1. \tag{3}$$

As by definition each "plate" multiplies the mol ratio of constituents by α, the mol ratio of constituents in the product is therefore

$$\left(\frac{x}{1-x}\right)\left(\alpha^{kN+1}\right), \tag{4}$$

the original evaporation in the still being equivalent to one plate in any case.

$\therefore$ Mol fraction of a in product

$$= x_p = \frac{x\alpha^{kN+1}}{1+x\left(\alpha^{kN+1}-1\right)}. \tag{5}$$

The liquid returned to the still is also not at equilibrium with the vapor and therefore differs from the liquid in the still. Its composition is evidently:—

$$x\left[\frac{\alpha}{1+x(\alpha-1)}\right]^k. \tag{6}$$

By the same method used in solving bubbling columns, *i.e.*, a balance of material entering and leaving the column, the vapor entering must therefore be equal to the product taken off plus the liquid returned to the still.

$$\therefore \quad \frac{xV\alpha}{1+x(\alpha-1)} = \frac{xP\alpha^{kN+1}}{1+x(\alpha^{kN+1}-1)} + x(V-P)\left(\frac{\alpha}{1+x(\alpha-1)}\right)^{k}, \tag{7}$$

$$\therefore \quad \frac{P}{V} = \frac{1-\left(\frac{\alpha}{1+x(\alpha-1)}\right)^{k-1}}{\alpha^{kN}-\left(\frac{\alpha}{1+x(\alpha-1)}\right)^{k-1}}. \tag{8}$$

While this is quite a complex equation which does not lend itself to further simplification, it may be evaluated for any particular case quite readily, preferably by inserting definite values for k; x and α being presumably known.

It will be noticed that the reflux ratio used differs somewhat from that usually employed, in that the product taken off is considered as a fraction of the total vapor produced. This has been found somewhat more convenient, but the more usual forms may be found quite readily; thus the fraction $\frac{V-P}{P}$ represents what is usually called the "reflux ratio".

The further assumption is also made that the molar heats of vaporization of the constituents are equal. This assumption is usually quite justified, but in some cases the difference is appreciable. This simply has the effect of changing the reflux ratio as the vapor ascends the column, but does not invalidate the equations if V is used to represent the mols of vapor passing upwards at any particular point.

Before using the above equations a few further useful relations may be noted for the theoretical case where $N=\infty$, as Equations 1 to 8 are all based on finite values of N.

When $N=\infty$, the product is completely pure for all values of $\frac{P}{V}$ less than a cricical value, while above this value impure product is obtained. The total amount of work then obtained from the column is finite, the efficiency factor, k, therefore zero. The same reasoning used in developing Equation 7 then gives:–

$$Vy = Px_p + x(V-P) \quad . \; . \; . \; . \; . \; . \; . \; . \quad (N=\infty), \tag{9}$$

or

$$\frac{P}{V} = \frac{y-x}{x_p-x} \quad . \; . \; . \; . \; . \; . \; . \; . \; . \; . \; . \quad (N=\infty). \tag{9a}$$

This equation is therefore the same as is found for a bubbling column (5, p. 599).

The maximum value of $\frac{P}{V}$ at which pure material may be obtained is given by substituting $x_p=1$, and the equation is meaningless at lower values of $\frac{P}{V}$.

Experimental Test of Equation 9 with a Very Effective Column

Rearrangement of 9a will show that even with an infinite column if the critical rate is exceeded the product simply consists of the pure product which might have been obtained diluted with *original liquid* up to the actual amount of product taken off.

This is a decidedly drastic conclusion, which so far as the author is aware is not emphasized anywhere in the literature and although it was in no way doubted, it was felt desirable to make an experimental test of the result. As a preliminary, Equation 8 was used to calculate the results available from columns where $N = 5$, 10, 20, 40 plates; and Equation 9a for $N = \infty$, when the original liquid consisted of 10% by weight of methyl alcohol in ethyl alcohol.

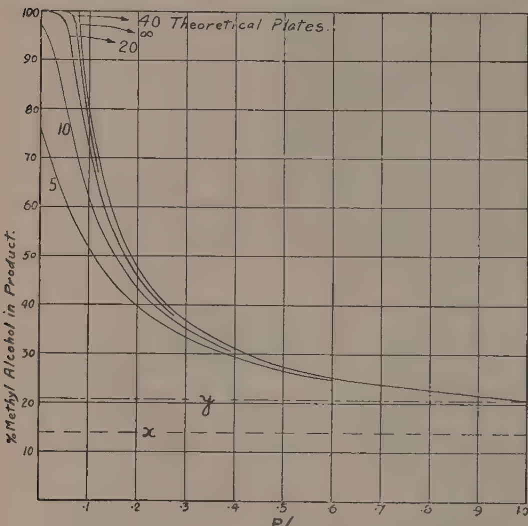

FIG. 1. *Distillation of ethyl alcohol + 10% by weight of methyl alcohol.*

For this case $\alpha = 1.635$, and $x = 0.1378$, and substitution of values of k from 0.05 to 0.95 gives Fig. 1. It will be seen that with $N = 5$ the highest concentration possible is only 75%, while the 40 plates give a result fairly close to the maximum possible, the purest material available, when $\frac{P}{V} = 0$, containing only 2.5×10^{-10} of ethyl alcohol.

This mixture was then distilled through a column which has been calibrated on the xylenes, pentane and isopentane, etc. This column gives a maximum number of plates in the neighborhood of 300, and for the present purpose should therefore be practically infinite.

The product ratio was set at the desired value and all product returned to the still continuously. The results obtained are given in Fig. 2, with the theoretical curve included.

In general the theoretical curve is followed, but the points immediately after the critical value are decidedly higher than is calculated. This must be due to a slight error in the assumptions at some point. It cannot be caused by any process inside the column, as such a large number of plates is effectively infinite and the results independent of the column. As the ratios were measured accurately the only remaining assumption which might be in error is that the vapor entering the column is the equilibrium vapor.

This is undoubtedly the source of this error, as it is quite a general phenomenon that the vapor evolved from an actively boiling liquid contains too much of the lightest constituent. This "error" is, in fact, the basis of one of the few successful methods so far applied to the separation of isotopes, the partial separation of mercury by distillation from a superheated surface, by Bronsted and Hevesy.

The importance of this fact cannot be overemphasized as practically all the vapor equilibrium data at present available have been determined by direct

boiling of the liquid under examination, and the statement is frequently made that such a process readily gives accurate results (5, p. 585.) This is unfortunately not the case, although the errors are certainly quite small in most of the work reported, and for the original purpose of the work probably negligible. This situation is entirely changed however when such data are used in calculating results of close fractionation.

That such small errors do exist even with systems studied repeatedly, and that their effect is by no means negligible can readily be seen by examination of data reported in (5, p. 600), which is an attempt to make calculation fit experimental results on the fractionation of ethyl alcohol from water. Examination of their Fig. 150 will show that, above the 30th plate, fractionation is actually proceeding more than three times as fast as the calculated curve shows. This is such a serious difference that even allowing for the fact that the experimental data are stated to be far from complete, it is still quite evidently impossible to increase the assumed plate efficiency, etc., enough to correct the error.

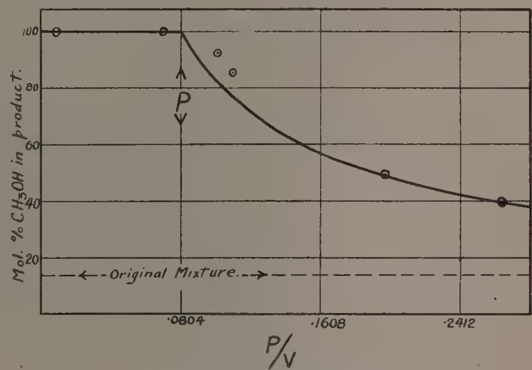

FIG. 2. *Distillation of alcohol mixture through nearly infinite column.*

In this connection reference might also be made to work reported by the author elsewhere (3) on the vapor equilibrium of glycerine solutions. In this investigation a great deal of time was spent investigating precisely this phenomenon, and it was found that gentle boiling was one of the worst methods of obtaining equilibrium vapor. The addition of a platinum "boiling coil" however reduces the error to nearly the minimum, but it still amounts in this case to about 2° C. The method found to give the closest approach to equilibrium was the use of a fairly concentrated source of heat in the form of a "boiling coil" combined with sufficient cooling below the liquid level to prevent too rapid evaporation, the whole apparatus being either thoroughly insulated or placed in a thermostat at exactly the equilibrium boiling point of the solution. With this arrangement it was found possible to reduce the error to slightly greater than 1° C., but the slight gain hardly offset the greater trouble, and the simpler "boiling coil" alone was used, as this error was eliminated further on in the apparatus.

In Table I are given a few unpublished results obtained during the progress of the work reported on glycerine solutions, t being the true boiling point of the glycerine solution at 66 cm. pressure, and Δt being the amount by which the vapor deviated from equilibrium; the solution with an equilibrium boiling

TABLE I

ERRORS RESULTING FROM DIRECT BOILING OF GLYCERINE SOLUTIONS, USING PLATINUM "BOILING COIL"

t, °C.	120	130	140	150	160	170	180	190	200
Δt, °C.	−2.4	−2.0	−4.0	−4.0	−7.5	−5.0	−6.5	−4.0	−4.0

NOTE:—*Abstracted from Ph.D. thesis, University of London, 1924.*

point of 120° C. giving a vapor which would be obtained at 117.6° C. under equilibrium conditions, and similarly with the others. Although this error appears rather small when compared with the difference in boiling points, it corresponds to a vapor containing only 83% of the required amount of glycerine.

In considering mixtures which boil much nearer together, the absolute error naturally decreases, and becomes more difficult to detect, but its relative importance remains approximately constant.

One very unfortunate feature of these small errors is that the application of Duhem's equation will not detect them. As this equation is based on the second law of thermodynamics it is a fundamental property, and even in the presence of serious systematic error, of the type considered, the equation is still satisfied.

The present paper is however not particularly concerned with the actual measurement of vapor equilibrium, beyond emphasizing the extreme degree of absolute precision required in such data before the results are of much value for the purpose of calculating the results of close fractionations, although it might be mentioned in passing that the apparatus suggested by Rosanoff (2) violates the phase rule, as three variables (vapor composition, pressure and temperature) are fixed on a divariant system. Unfortunately the method does not eliminate errors and the original vapor might have been condensed directly.

Possibility of Saving Time in a Fractionation

In starting any particular distillation it is obviously desirable to obtain the result as rapidly as possible. The faster a distillation is performed however the more often it must be repeated, and some particular speed is evidently the most economical.

The work to be done is obviously proportional to the number of mols to be distilled, and may also be considered proportional to the number of theoretical plates, operating at $\frac{P}{V}=0$, which would be required to give the desired separation. The work obtained from any particular distillation is evidently some fraction of this number of plates, and is therefore equal to $kN+1$, and the time spent obtaining one mol of product is proportional to $\frac{V}{P}$.

Combination of these data gives the relative time spent per mol of product, per plate of separation as:—

$$\frac{1}{kN+1}\cdot\frac{V}{P}\,. \qquad (10)$$

However, several factors must still be considered before evaluating such an equation, as repeated distillation has several definite disadvantages.

1. It is never proportionately more effective, some separation being inevitably lost in the process.
2. Losses of material by evaporation, etc., are more serious.
3. More work is entailed in analyzing samples, recording and co-ordinating data, etc.
4. The curves obtained from a redistillation are never as smooth as the first obtained.

All these effects tend to give more weight to the first fraction of (10) than the last, and it does not seem too much to use the $\frac{3}{2}$ power, giving the relative time spent in producing a result as:—

$$\frac{1}{(kN+1)^{\frac{3}{2}}} \cdot \frac{V}{P}, \text{ per "plate", per mol.} \qquad (11)$$

The $\frac{3}{2}$ power is of course estimated, but the second power is definitely too high.

Using this equation the relative times spent treating the methyl-ethyl alcohol mixture considered in Fig. 1 were calculated for each column, and over the useful range of $\frac{P}{V}$.

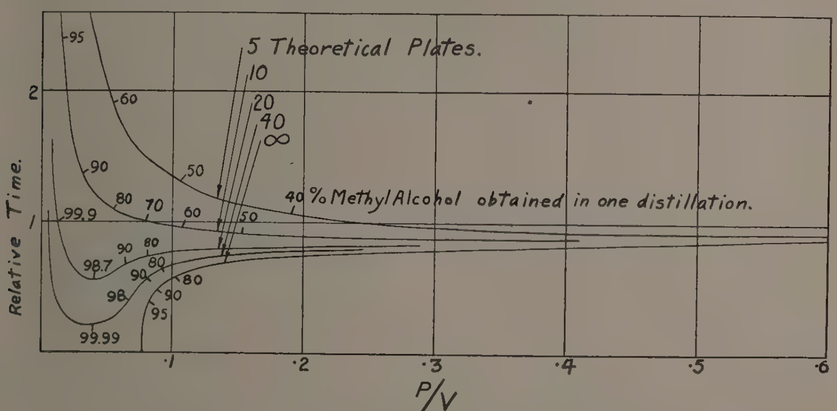

FIG. 3. *Relative times spent distilling alcohol mixture.*

The results of this calculation are given in Fig. 3 in which the times spent per mol distilled, per "theoretical plate" of separation, are plotted against the ratio $\frac{P}{V}$, for each column.

It will be seen that the infinite column theoretically consumes no time at all values of $\frac{P}{V}$ below the critical value (0.08038). This is simply due to the fact that it produces completely pure product at any value of $\frac{P}{V}$ less than the critical, which always represents an infinite number of plates, and the time

consumed per plate separation is therefore zero. It is of interest however to note that if the critical value is exceeded by only 0.00005, raising the ratio to 0.08043, the infinite column is reduced to the equivalent of only 21 theoretical plates. This is an extremely drastic reduction, which is not in any way compensated by the slightly increased rate.

With the 40- and 20-plate columns a sharp maximum of efficiency (minimum of time) is obtained at $\frac{P}{V} = 0.04$. This it will be seen is a half of the "critical value".

However, the actual value of $\frac{P}{V}$ used in practice with these columns would be somewhat affected by other considerations, principally the fact that only one distillation is required to give the desired result, and the reflux ratio is therefore set at the *maximum rate* which will still permit sufficiently pure product to be obtained. The purity of the product obtained in one distillation is marked on these curves.

With the 10- and 5-plate columns a still different type of result is obtained, in that the columns are somewhat inadequate, and a decrease of $\frac{P}{V}$ to low values is therefore not compensated by a corresponding gain in purity. These columns however do show a slight minimum time at $\frac{P}{V} = 0.4$ and 0.6 respectively, and in cases where the saving of time or money is the principal factor these columns should be used at about those values of $\frac{P}{V}$, but in the laboratory the saving of material and human inertia would combine to suggest that these columns be used at the *lowest value* of $\frac{P}{V}$ which does not introduce serious loss of efficiency. This process combines the maximum amount of separation per distillation with the minimum number of treatments. In the laboratory the "10-plate" column would probably be best used at $\frac{P}{V} =$ about 0.08, giving 69% product in one treatment, while the "5-plate" column is probably most economical at about $\frac{P}{V} =$ about 0.14, giving 45.5% product the first time.

It will be seen that in the above cases all of the best values of $\frac{P}{V}$ are closely related to the critical value for the infinite column, and in fact only in two cases, when x is very low, and when the column is somewhat inadequate, is it an economy of time to depart very appreciably from this value.

The more general problem may be considered under the following groups: *$x = 0.2$ or higher.* With an adequate column it is not economical to hurry the process, but to use the highest value of $\frac{P}{V}$ which will give the desired product, usually somewhat less than the "critical value"; while if the column is less efficient a small amount of time may be saved by distilling more rapidly, but the saving is never great.

$x =$ less than 0.2. As the concentration of the desired constituent in the original mixture is decreased below 0.2, it becomes more economical to distil

at greater rates, especially if the column is not particularly efficient. The lower x becomes, the lower is the critical value and the greater the economy which may be obtained by distilling at rates greater than the critical. This is the principal case when time may be saved by departing from the critical value. As an approximate guide it is suggested that if $x=0.05$ or less the critical value should be exceeded by the factor $\sqrt{\frac{1}{x}}$.

The difference in boiling point of constituents seems to make very little difference to the above conclusions, although as the boiling points are closer the critical value of $\frac{P}{V}$ naturally decreases (see Equation 9a when $x_p=1$) and the rate of distillation must be correspondingly decreased. The above results are summarized in Table II.

TABLE II

FACTOR BY WHICH CRITICAL VALUE OF $\frac{P}{V}$ FOR INFINITE COLUMN SHOULD BE MULTIPLIED FOR MOST ECONOMICAL WORK

x	Adequate column	Moderately adequate column	Inadequate column
0.2 or higher	0.5 to 1	0.25 to 0.5 or 1 to 1.5	1.5 to 2.5 or more as the column becomes less efficient
0.05 to 0.2	0.5 to 1	0.25 to 0.5 or 1 to 2	2 to 3 or more, as above
Less than 0.05	$\sqrt{\frac{1}{x}}$	$\sqrt{\frac{1}{x}}$	$\sqrt{\frac{1}{x}}$

For the moderately adequate column take the top figure if the column will just give the desired result in one distillation; if two are needed take the lower; while if more than two, consider the column inadequate.

It remains only to evaluate the critical value of $\frac{P}{V}$ for the infinite column for any particular case, to be able to decide at once the most economical value for any laboratory distillation. This calculation has been made for ideal mixtures of constituents which boil from 0.25° to 32° C. apart, and the result plotted on a double logarithmic scale over the range of $x=0.001$ to 1.0, giving Fig. 4.

These values are all calculated for what is considered a "normal" liquid, *i.e.*, one which gives a value of 1.20×10^{-4} in Craft's equation for the correction of boiling points to normal pressure. If the liquids have a lower value than 1.2×10^{-4}, *e.g.*, the alcohols, they are somewhat more readily separated and a proportionately higher rate may be used; while in the reverse case, *e.g.*, chlorinated hydrocarbons, etc., the rate should be correspondingly reduced.

A further assumption was also made that the lighter constituent boils at

100° C. The values of Δt given at the right of the plot are directly proportional to the absolute boiling point of the lighter constituent, and should therefore be correspondingly increased or decreased if it boils materially higher or lower than 100° C.

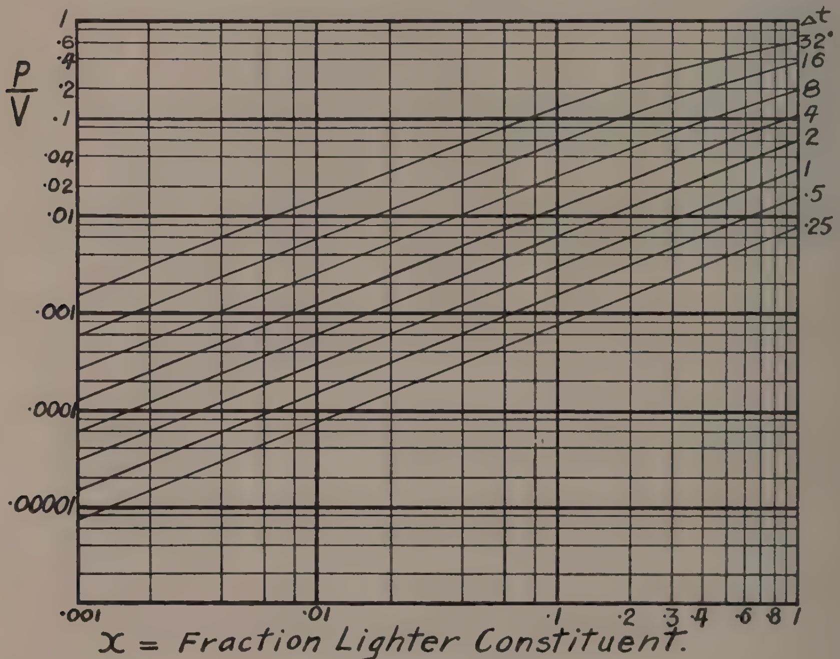

FIG. 4. *Critical values of* $\frac{P}{V}$ *for infinite column.*

A further trouble is, of course, encountered in using these data in the laboratory, in that very few mixtures can be considered ideal. If the deviation is at all serious, accurate calculations are impossible unless the system be studied previously. For a guide in such separations the boiling points of mixtures should be investigated if possible, as the tangent to the boiling point curve, at the concentration considered, extended to the axes, gives a very good estimate of the behavior of the mixture.

When these calculations are made it is rather surprising that the critical values obtained should be so low, *e.g.*, even with a mixture containing 8% of a constituent boiling 32° C. below the remainder, 90% of the vapor distilled into the column must be returned as liquid. This is a much higher reflux ratio than is usually adopted in the laboratory for distilling such a mixture (1, p. 366).

In order to apply the above data to any distillation the following steps are necessary.

1. Calibration of the column, or at least a fairly close idea should be obtained of what separations may be obtained with it.

2. Consideration of the mixture itself, whether it is nearly ideal or otherwise. Known data on compounds chemically similar should be consulted if necessary.

3. The boiling points of the constituents are also required, and at least approximate concentrations.

4. From Fig. 4 the critical value of $\frac{P}{V}$ for the infinite column is obtained for the particular mixture.

5. From Table II suggestions may be obtained giving the amount of departure from the critical value likely to result in the greatest economy.

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A SIMPLE APPARATUS FOR PURIFYING RADON[1]

By G. H. Henderson[2]

Abstract

A description is given of a simple and efficient apparatus for purifying radon, which has given several years effective service in connection with radium therapy. Potassium hydroxide is used to remove carbon dioxide and most of the water. Since phosphorus pentoxide for drying has been dispensed with, only one Toepler pump and fewer valves and stopcocks are necessary. The number of stopcocks has been reduced to three and there is but one large bulb and one float valve. The impurities are removed by sparking and allowing excess hydrogen to escape through a palladium tube heated electrically; thus there is no danger of clogging the tap with particles of loose reagent. All parts are made of Gundlach glass. Purification takes from two to five minutes.

In radium therapy, radon is being used more and more widely in place of the radium salt because of its many advantages. When radium is used in this way some form of plant is necessary for pumping off, purifying and tubing the radon. Many descriptions have been published of apparatus designed to fulfil this requirement. While serving their purpose, these plants have generally been very complicated with numerous bulbs, float valves and stopcocks (the latter alone usually numbering eight or more). Highly trained personnel is consequently required to operate the plant. The dangers of breakage and of clogging of valves and stopcocks are ever present in using glass apparatus. These dangers are multiplied in proportion as the apparatus is more complicated.

It seemed feasible to design a greatly simplified apparatus which would give equally good results. This apparatus should be cheaper, less liable to breakage and more easily manipulated than the usual type. These features are very desirable if the use of radon is to be extended so that small centres of population could enjoy its benefits.

Such an apparatus was developed in this laboratory with the aid of a grant from the National Research Council of Canada and a description of it published by W. G. Moran (1, 2). After the experimental-plant, set up in the Victoria General Hospital, Halifax, had been in use for three years with very satisfactory results, it became necessary to remove it to another part of the hospital. Advantage of the opportunity was taken to rebuild the plant, incorporating small improvements and paying more attention to appearance. A short description of the plant follows. Many details which have already been sufficiently described in Moran's paper are omitted.

Description of Plant

The method of operation will be clear from a consideration of Fig. 1. The radon is pumped from the radium solution in the flask, 1, by the Toepler pump, 2, into the purifying unit, 3, which had been previously evacuated through the

[1] *Manuscript received August 1, 1931.*
Contribution from the Department of Physics, Dalhousie University, Halifax, N.S.

[2] *Professor of Mathematical Physics, King's College, Dalhousie University.*

stopcock, 6. The impurities are removed by sparking and allowing the excess hydrogen to escape through a palladium tube heated electrically from the outside. A little potassium hydroxide fused to the wall of the unit removes carbon dioxide and most of the water. When purification is complete the radon is simply pushed up into the capillary tube above and sealed off ready for use.

It will be seen that the number of stopcocks has been reduced to three, all of which can be cleaned without admitting air to the solution. There is but one large bulb and one float valve.

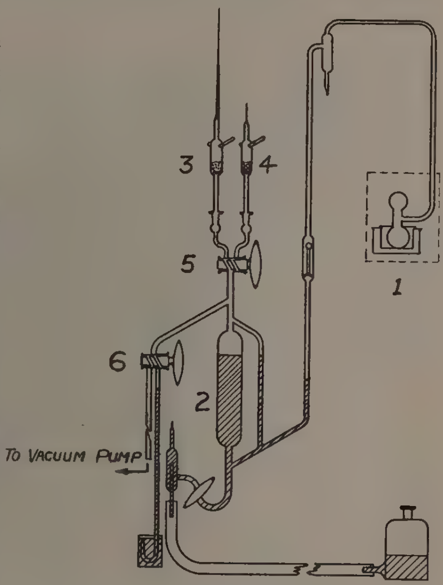

FIG. 1. *Diagram of apparatus for purifying radon.*

It will be obvious to those familiar with the different types of radon plants that this simplification has been obtained by dispensing with the usual phosphorus pentoxide for drying the radon and by the use of palladium. Both of these features are original. The palladium method substitutes a clean physical purification for a chemical one involving frequent renewals of reagents and demanding great care lest the internal heating arrangements burn out. The use of pentoxide has always necessitated a second Toepler pump and numerous valves and stopcocks in order to transfer the purified radon into the capillary. In the present arrangement the radon is pushed straight through the purification unit into the capillary with a minimum of handling. Most of the water impurity is taken up by the potassium hydroxide. The remainder condenses on the walls of the unit as the mercury moves up. A visible layer of condensed water has never been observed on the top of the mercury in the capillary.

An alternative purifying unit, 4, is shown in Fig. 1 which is useful, though not necessary, in preparing gold "seeds". The tube leading from stopcock 6 to the pneumatic trough is not necessary for the process described above, but is used for transferring radon elsewhere if required.

The completed plant is shown in the photograph, Fig. 2. The framework is made of $1\frac{1}{4}$ in. pipe using stock fittings and angle irons. Provision has been made for a duplicate installation. The radium solution is kept in a commercial safe and is surrounded by over half a ton of lead bricks, giving an average protective thickness of more than five inches. The tube leading to the Toepler pump is protected by lead tubing of $\frac{3}{4}$ in. wall thickness. The safe and contents are kept cooler than the room by circulating water, thus preventing distillation of the solution into the pump.

The purification unit is evacuated through stopcock 6 by a Hyvac pump. With an occasional cleaning this pump has given a satisfactory vacuum for over three years.

All glass parts of the apparatus are made of Gundlach glass. Attention is drawn to the shape of the flask containing the solution. The upper bulb is for the protection of the solution in case of an inrush of mercury. Against this remote contingency three safeguards have already been provided: (1) a float valve, (2) a trap and (3) by having the tube leading to the solution rise to more than barometric height above the top of the Toepler pump. Should mercury reach the flask in spite of these safeguards the solution would be displaced into the upper bulb of the flask and held safely there. The contingency, as already remarked, is remote but is present in all types of radon plant.

FIG. 2. *Photograph of plant for purifying radon.*

Performance

This type of apparatus has given satisfactory results for over three years. The radon can readily be concentrated to 100 millicuries per cubic millimeter. This would mean, for instance, 10 mc. in a gold "seed" 5 mm. long and 0.15 mm. internal diameter. The apparatus works quickly, the actual purification taking from two to five minutes.

It might be thought that trouble would arise since no particular pains are taken to remove water vapor. In particular there might be fear of occasional blow-outs in sealing off the capillary. No such trouble has developed. Only three blow-outs have occurred since the plant was set up, all caused by attempting to seal off tubes at too high gas pressures. There cannot be more water vapor in the compressed radon than the amount corresponding to the water vapor pressure at room temperature.

The alternative method of purifying by hot copper oxide, described by Moran, has been given up and the palladium tube method used exclusively.

The latter is a purely physical method which is indefinitely reproducible and does not involve any danger of clogging the capillary with particles of loose reagent.

The amount of radium in solution in this apparatus in the Victoria General Hospital is 200 mg. There seems to be no reason why it should not work equally well with larger quantities of radium.

Acknowledgment

The author wishes to thank Mr. S. T. Alvey for the skill and care with which he has constructed the plant.

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THE ACTION OF HIGH-SPEED CATHODE RAYS ON THE SIMPLER ALCOHOLS, ALDEHYDES AND KETONES, AND ON ETHYLENE[1]

By J. C. McLennan[2], F.R.S. and W. L. Patrick[3], Ph.D.

Abstract

The results are set forth of an experimental examination of the action of high-speed electrons on the vapors of acetaldehyde, acetone, methyl alcohol and ethyl alcohol, on gaseous formaldehyde and on ethylene. It has been shown that cathode rays form polymerization compounds with formaldehyde, acetaldehyde and acetone and that these suffer decomposition into the simpler gases. The ultimate decomposition of acetaldehyde has been studied in some detail and has been shown to proceed through the polymer. Exposure to the rays results in the decomposition of methyl and ethyl alcohols with the formation of aldehydes, hydrocarbons, oxides of carbon and hydrogen. The conclusion has been drawn that dehydrogenation is the principle primary reaction in the decomposition of the alcohols, further decomposition proceeding through the aldehydes. Ethylene under the action of the rays yields an unsaturated liquid hydrocarbon which upon further bombardment gives hydrogen, acetylene and saturated hydrocarbons.

Introduction

High-speed electrons have been used in studies of the ammonia equilibrium (3, 9), of the polymerization of acetylene (10) and of the oxidation of hydrogen (7), methane and carbon monoxide (8). Other investigations have been concerned with the formation of ozone from oxygen (1), and with the effects of the rays on air, nitric oxide and carbon dioxide (7). The present contribution deals with the changes which occur when the simpler alcohols, aldehydes and ketones, and also ethylene, are subjected in the gaseous state to high-speed electronic bombardment.

Losanitsch (5, 6) performed experiments in the silent electric discharge with the vapors of several substances, including methyl and ethyl alcohols and acetaldehyde. Poma and his collaborators (14, 15) passed the vapors of various alcohols and acetone through an intense discharge from an induction coil and also through a silent discharge. Decomposition was observed in every case, sometimes accompanied by the formation of liquid products. The present investigation has shown that high-speed electrons are capable of decomposing organic compounds; in some cases decomposition proceeds through an intermediate polymeric compound, but with the alcohols dehydrogenation appears to be a favored reaction.

Description of the Apparatus

The cathode ray tube was operated by a transformer system similar to that described by Coolidge (2), Fig. 1. The mains of the 110 volt, 50 cycle current were connected in series with a variable resistance (A), an ammeter (B) and

[1] *Manuscript received August 4, 1931.*

Contribution from the Physical Laboratory of the University of Toronto, Canada, with financial assistance from the National Research Council of Canada and the Carnegie Corporation of New York.

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the primary of a 2:1 transformer (C). The secondary of the transformer supplied current to the primary of a 1:1 insulation transformer (D) having a secondary insulated for 125 kv. The primary of a 200-kv. Snook transformer (E) was fed by the secondary of transformer D. The case of the high tension transformer was insulated from earth and connected to the primary and to the middle of the secondary. One terminal of the secondary was connected to the cathode of the tube and the other was joined to earth through a milliammeter (F). This method of operation was a convenience, since the window of the cathode-ray tube and any apparatus connected to it could be grounded. The window was made of resistal (nichrome steel) foil and was soldered to the brass end-piece.

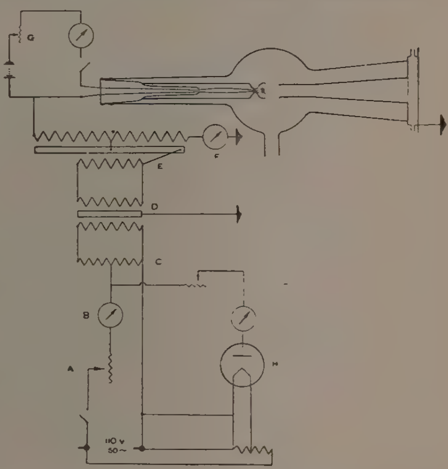

FIG. 1. *The electrical circuit.*

The tungsten filament was heated by a battery of about 12 volts; this battery, an ammeter and the variable resistances (G) were placed in a galvanized iron box, the corners and edges of which were rounded in order to cut down corona discharge. This box was placed on a suitably insulated stand. The current through the filament was controlled by means of two ebonite rods which passed into the box and which were connected to the variable resistances. These were such as to provide a coarse and a finer adjustment.

In order to decrease the voltage of that half of the wave not utilized by the cathode-ray tube and thus to bring the inverse voltage below the "useful", a Tungar valve (H) was connected in parallel across the primary of the transformer C. The voltage applied to the tube was calibrated by means of a standard Victor X-ray spark gap.

The reaction chamber, of a little more than a litre capacity and made of Pyrex glass, was waxed into an annular groove in the brass end-piece, which in turn was sealed by means of wax to the glass of the tube. A stream of water from a thermostat maintained at 20° C. passed through a copper spiral wound round the reaction cell and lagged on the outside, and then through a jacket which served to keep the window at constant temperature and the vacuum-tight wax seals hard. During an experiment, the cathode-ray tube was continuously exhausted through a liquid air trap by means of a mercury-vapor diffusion pump and an auxiliary Hyvac oil pump.

The reaction chamber was connected to a manometer (M), a mercury-filled gas sampling eudiometer, a Sprengel pump (S), and through a side tube to a Hyvac oil pump (P) and to the gas and liquid reservoirs (Fig. 2). The system was constructed throughout of Pyrex glass. Three-way taps permitted of the

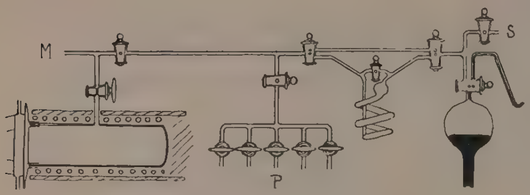

FIG. 2. *The reaction system.*

inclusion of a glass spiral between the reaction chamber and the sampling apparatus; thus the gaseous mixtures could be bubbled through water or passed through a liquid air trap as occasion demanded.

The Initial Substances

Each liquid was obtained in the purest possible form and distilled, an intermediate and constant-boiling distillate being collected in the small vaporizing bulb. This was sealed to the apparatus; subsequently, only vapor at the temperature of the laboratory was allowed to enter the reaction system.

Gaseous formaldehyde was prepared in a similar bulb by heating dried paraformaldehyde. The carbon monoxide and hydrogen used in the experiments with acetaldehyde were prepared in a high state of purity by the recognized methods, washed by suitable reagents, dried by passage over phosphorus pentoxide and stored in two-litre glass reservoirs sealed to the reaction system. Methane was prepared by heating a mixture of anhydrous sodium acetate and barium hydroxide, and purified by passage through glass worms containing fuming sulphuric acid, dilute nitrochromic acid and caustic potash solution. Ethylene was prepared by heating one part of ethyl alcohol with four parts of concentrated sulphuric acid and purified by bubbling through caustic potash solution and concentrated sulphuric acid. Each hydrocarbon was dried by passage over phosphorus pentoxide and liquefied in a liquid air trap. On subsequent distillation, the middle fraction was stored in the aforementioned manner.

Experimental

Prior to any series of experiments the reaction chamber was evacuated; evacuation was continued for several hours with the chamber under bombardment. In this way, the glass was thoroughly outgassed.

The train of tubes leading to the reaction chamber was evacuated and swept out twice with the gas or vapor under investigation. This was repeated with the reaction vessel. Vapor could then be admitted to any desired pressure below that of saturation. The bombardment was commenced and allowed to continue for a definite period, pressure readings being taken at known intervals. The arrangement of the apparatus permitted of the withdrawal of liquefiable gases to a liquid air trap. In this way the partial pressure of any 'permanent gas' formed during an experiment could be measured. Water could be admitted to the trap and later, both gaseous fractions removed for analysis. This permitted of greater ease in the detection of condensable resultants.

After other bombardment experiments with the organic vapors, samples of the resulting gaseous mixtures were withdrawn through 5 cc. of distilled water,

contained in the spiral, and allowed to stand before analysis for several days over mercury in the presence of sticks of zinc chloride. All gas analyses were carried out in a Bone-Newitt gas analysis apparatus, which had been supplemented with a copper oxide tube for fractional combustions. The analyses are recorded on a nitrogen-free basis, adventitious nitrogen being in every case less than 1%. Unless otherwise stated, it may be taken that the values of the applied voltage and tube current during bombardment were 136 kv. and 0.065 milliamperes respectively.

Results

In all, seven series of experiments were carried out, the substances bombarded being gaseous formaldehyde, solid paraformaldehyde, the vapors of acetaldehyde, acetone, methyl alcohol and ethyl alcohol, and finally, ethylene.

Series I and II

Gaseous formaldehyde was allowed to enter the reaction chamber to a pressure of 300 mm. The autopolymerization curve was obtained by plotting pressure against time (curve ABC, Fig. 3). The gas was again admitted, but when the pressure had fallen to 220 mm., bombardment was commenced. The pressure fell rapidly to a minimum value, after which it increased continuously with prolonged exposure to the rays (curve ABDE). The resulting gaseous mixture was freed from aldehyde vapor and analyzed. This procedure was repeated until a point (F) halfway down the sharp fall in pressure was reached, when the bombardment was discontinued and the curve of autopolymerization plotted (curve ABFG). During the course of these experiments, a white solid was deposited on the walls of the reaction chamber. This had been formed by the combined actions of autopolymerization and bombardment. The apparatus was evacuated and the solid subjected to the action of the rays (curve MN) for the same period (250 min.) as in the first and prolonged bombardment experiment, when the resulting gaseous mixture was withdrawn for analysis. The gas analyses of these series are recorded in Table I. No traces of unsaturated hydrocarbons could be detected in these mixtures. The solid removed from the reaction chamber at the end of these experiments was free from carbon.

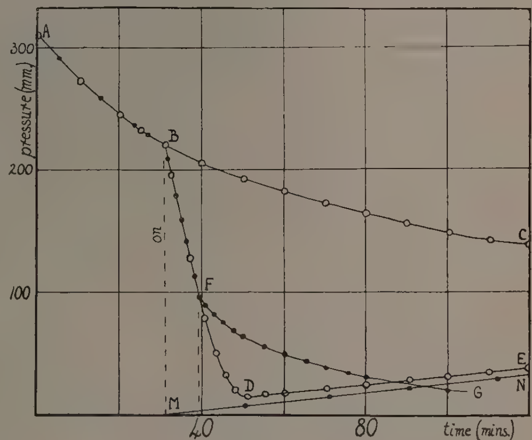

FIG. 3. *The curves of Series I and II.*

Series III and IV

In these experiments gaseous acetaldehyde and acetone were bombarded at various pressures for known time intervals, the products being withdrawn for

TABLE I

COMPOSITIONS OF THE GASEOUS MIXTURES OBTAINED FROM EXPERIMENTS WITH FORMALDEHYDE

Initial state	Final pressure in mm.	Gaseous mixture			
		CO_2 %	CO %	H_2 %	CH_4 %
Gaseous formaldehyde	69.0	23.6	29.4	39.6	7.4
Paraformaldehyde	68.0	28.0	25.3	40.0	6.7

TABLE II

PERCENTAGE COMPOSITIONS OF THE GASEOUS MIXTURES FORMED DURING THE DECOMPOSITIONS OF ACETALDEHYDE AND ACETONE

Substance	Acetaldehyde		Acetone	
Initial pressure in mm.	190	40	105	105
Time of exposure in hours	4	4	2	4
CO_2	4.2	5.5	7.4	8.1
C_2H_2	1.9	1.3	1.3	1.1
C_2H_4	1.3	1.0	1.2	1.2
CO	37.8	37.2	30.1	29.9
H_2	37.6	39.3	21.5	25.3
CH_4	14.7	13.2	18.0	15.3
C_2H_6	2.5	2.5	20.5	19.1
C/A range (saturated hydrocarbons)	1.79–1.86		1.41–1.49	

analysis. The results are incorporated in Table II, in which a comparison is made of the values of C/A (the ratio of the contraction to the amount of carbon dioxide formed on explosion with excess of oxygen) obtained for the saturated hydrocarbons after the removal of hydrogen, oxides of carbon and unsaturated hydrocarbons. The ranges given include the values from several analyses, usually five or six. From these values, the saturated hydrocarbons were calculated as methane and ethane.

Whereas the bombardment of acetone was always accompanied by an increase in pressure, the sign of the pressure effect with acetaldehyde was influenced by the magnitude of the initial pressure. In an experiment with the initial pressure of acetaldehyde at 190 mm. the rate of change of pressure was negative, indicating that the bombardment was inducing some polymeric change. A yellowish liquid was found to collect within the reaction cell. Eventually after three hours bombardment the pressure reached a minimum value, increasing slightly with further exposure to the rays. On circulating the final gas through the liquid air trap, a residual gas was obtained containing carbon monoxide and hydrogen. The experiment was repeated in order that a complete analysis of the gaseous products might be made. From such data, it was possible to deduce that at pressures in the neighborhood of 100 mm. little change in pressure would be caused by bombardment. In subsequent experi-

ments it was found that no appreciable diminution in pressure occurred with acetaldehyde at an initial pressure of 90 mm.; below this value, however, the pressure was increased.

At this stage the question of the kinetics of these reactions became of importance. Experiments were conducted with acetaldehyde to determine the various influences of (a) the initial pressure, (b) the decomposition products and (c) the applied voltage on the rate of change of pressure at low initial pressures. The pressure-time curves obtained in these experiments were straight lines. The results are recorded in Tables III, IV and V. At voltages below 80 kv. no change could be detected in the contents of the reaction vessel. This, then, was the approximate voltage to be applied to the tube before any electrons passed through the resistal metal window. Above this value, increase in applied voltage resulted in an increase in the rate of decomposition.

TABLE III

INFLUENCE OF INITIAL PRESSURE ON THE RATE OF CHANGE OF PRESSURE OF ACETALDEHYDE DURING BOMBARDMENT

Initial pressure in mm.	4.9	10.8	21.9	24.4	32.5	42.8
dp/dt, mm. per min.	0.020	0.018	0.018	0.018	0.019	0.020

TABLE IV

INFLUENCE OF THE DECOMPOSITION PRODUCTS ON THE RATE OF CHANGE OF PRESSURE OF ACETALDEHYDE DURING BOMBARDMENT

Partial pressure of acetaldehyde in mm.	40.0	40.9	39.5	39.5
Diluent	—	H_2	CO	CH_4
Percentage diluent	—	21.4	19.7	20.6
dp/dt, mm. per min.	0.020	0.022	0.017	0.022

TABLE V

INFLUENCE OF APPLIED VOLTAGE ON THE RATE OF CHANGE OF PRESSURE OF ACETALDEHYDE DURING BOMBARDMENT, THE INITIAL PRESSURE BEING 40 MM.

Applied voltage in kv.	80	90	113	136	160
dp/dt, mm. per min.	0	0.006	0.016	0.020	0.025

After these experiments with acetaldehyde, and those of Series IV with acetone, the respective condensates were removed for examination. In view of the small quantities obtained, this was effected by means of a glass rod. The viscosities increased on standing in air. This effect was more marked in the case of the condensate from acetaldehyde than in that from acetone; otherwise no distinction could be made between the two liquids. They were miscible in chloroform and the solutions thus prepared did not decolorize

bromine. On extraction with water, they yielded a white wax-like solid. It would appear that the condensates are mixtures of this solid and the initial substances, and that the solid is a saturated polymer of a substance capable of being present from either acetaldehyde or acetone, that is, a polymer of formaldehyde or of acetaldehyde. The pressure effects during these experiments are in favor of the latter.

Series V and VI

The vapors of methyl and ethyl alcohols were subjected to bombardment. The pressure increased in every experiment. Neither liquid nor solid products could be detected in the reaction chamber after the experiments with methyl alcohol, but with ethyl alcohol there were slight traces of the presence of a yellow oil. The amount was, however, too minute for removal. The analyses of the gaseous decomposition products are recorded in Table VI.

The aqueous extracts of the resultant gaseous mixtures after experiments in Series IV, V and VI were removed from the spiral and tested for aldehydes. Formaldehyde was present in every case. Quantitative estimations were conducted for the relative amounts of formaldehyde and acetaldehyde present during the decompositions of methyl and ethyl alcohols, the initial pressures being 50 mm. and 28 mm. respectively. Romijn's cyanide method was used for the determination of formaldehyde and Ripper's bisulphite method for total aldehydes. Acetaldehyde was then found by difference. These methods have an accuracy of the order 1 in 100,000 and are uninfluenced by the

TABLE VI

PERCENTAGE COMPOSITIONS OF THE GASEOUS MIXTURES FORMED DURING THE DECOMPOSITIONS OF METHYL AND ETHYL ALCOHOLS

Alcohol	Methyl		Ethyl	
Initial pressure in mm.	51.3	48.4	27.8	28.0
Time of exposure in hours	2	4	2	4
CO_2	9.8	10.9	10.6	10.0
C_2H_2	0.2	0.0	1.4	0.7
C_2H_4	0.6	0.6	2.4	2.1
CO	21.2	22.0	18.5	19.1
H_2	57.0	56.2	47.5	47.8
CH_4	11.2	10.3	16.6	17.1
C_2H_6	—	—	3.0	3.2
C/A range (saturated hydrocarbons)	1.93–1.97		1.7–1.8	

presence of the alcohols in dilute solution. The results are incorporated in Table VII. For purposes of comparison it is convenient to express these results in terms of the original α-carbon atoms recovered as aldehydic carbon. No appreciable difference could be distinguished between the contents of the solutions obtained with the same alcohol after two or four hours exposure to the rays.

Series VII

Bombardment of ethylene results in a fall in pressure with the formation of a liquid product. The rate of change of pressure increases with the initial pressure (see Table VIII) but the relation is not a linear one; the reaction order is greater than unity, indicating that two or more molecules of ethylene are involved. In any experiment the rate of change of pressure falls off more rapidly than would be expected from the change in total pressure. It may be supposed from this that other gases are being formed during the bombardment. The detection of acetylene and of a 'permanent' gas verified this supposition. In later experiments the liquid condensate was bombarded; a gas of the composition $C_2H_2 = 10.7$, $C_2H_4 = 4.5$ and $H_2 + CH_4 + C_2H_6 = 84.8\%$ was liberated, the mixture of hydrogen and saturated hydrocarbons possessing the C/A value of 3.3. The corresponding mixture obtained by bombarding ethylene returned values for this ratio in the neighborhood of 2.2, indicating a lower hydrogen content together with the possibility of the hydrogenation of the ethylene. The analyses were, however, not carried further.

TABLE VII

AMOUNTS OF ALDEHYDES FORMED DURING THE BOMBARDMENT OF METHYL AND ETHYL ALCOHOLS

Alcohol	Time in hours	Weight —CHO in mg. recovered as		Per cent original α-carbon recovered as aldehydic carbon in	
		H.CHO	CH_3.CHO	H.CHO	CH_3.CHO
Methyl	2	2.0	1.8	2.5	2.25
	4	2.0	1.9	2.5	2.4
Ethyl	2	0.6	1.2	1.35	2.7
	4	0.6	1.2	1.35	2.7

TABLE VIII

INFLUENCE OF INITIAL PRESSURE ON THE RATE OF FALL IN PRESSURE OF ETHYLENE DURING BOMBARDMENT

Initial pressure in mm.	84	140	260	400
dp/dt, mm. per min.	12	28	56	128

Mund and Koch (11, 12, 13) and more recently, Lind, Bardwell and Perry (4), have described a colorless liquid obtained by the action of α-particles on ethylene, likening the odor to that of petroleum or of turpentine. The oil obtained in these experiments with high-speed electrons was discolored by carbon and burnt with a smoky luminous flame; it had a high viscosity and a low vapor pressure. The strong odor was similar to that characterizing the higher unsaturated hydrocarbons. The condensate was insoluble in water but readily and completely soluble in chloroform. An apparent partial solubility

in alcohol suggested the presence of two or more components. The color change attendant upon the addition of potassium permanganate in alcoholic solution and also of bromine in chloroform, gave strong evidence of unsaturation.

Discussion of the Results

The pressure-time curves set forth in Fig. 3 enable the conclusion to be drawn that the outstanding action of cathode rays on gaseous formaldehyde is one of polymerization. Bombardment produces a sudden fall in pressure. Cessation of bombardment (as at F) is accompanied by a rapid falling-off in the rate of pressure change. These facts give rise to the view that the rays promote the formation of molecular clusters, which separate from the gas phase as a white solid on the walls of the reaction vessel. Prolonged bombardment resulted in the formation of a residual gas, which subsequent experiments showed to be due to the decomposition of the white polymer. Curve MN almost coincides with curve DE in later stages. The results of the gas analyses given in Table I show that the decomposition is not the simple process which might be expected from single molecules of formaldehyde, but is a more intricate one, involving the breaking up of molecular complexes to yield simple gaseous products.

The straight-line pressure-time curves, the independence of the speed of decomposition of initial pressure (Table III) and the negligible effects of large initial partial pressures of the resultants (Table IV) together establish the fact that the decomposition of gaseous acetaldehyde at initial pressures below 40 mm. is a reaction of zero order unretarded by the resultant products. It may be concluded that within this experimental range and for this value of the applied voltage (136 kv.), at least, the decomposition reaction occurs on the walls of the reaction vessel.

The results given in Table V show that the rate of change in pressure increases with increase in applied voltage; when the latter exceeds 100 kv., however, the rate of change increases less rapidly. This appears to indicate that at voltages above 100 kv., when the gaseous pressure is 40 mm., by far the greater number of electrons are moving with velocities sufficiently large to enable them to reach the walls of the vessel before suffering collisions which are effective in producing molecular disruption. Below this value, the rate of change in pressure is proportional to increase in voltage since the number of effective collisions in unit time in the gaseous enclosure will be proportional to the speed of the electrons and above it because the proportion of the electrons, in a beam of varying velocities, reaching the walls will increase slightly with increase in the energy of the beam. These facts support the conclusion given in the previous paragraph.

The experiments of Series III and IV show that both acetaldehyde and acetone are capable of giving condensates when bombarded and that this effect is more predominant with the former. The analyses incorporated in Table II show that the decomposition products are independent of the pressure and that they cannot be explained by a simple equation. These facts, to-

gether with those established in the kinetic experiments, give rise to the view that the rays promote the formation of clusters as in the case of formaldehyde, these subsequently undergoing decomposition on the walls of the vessel into the simpler gases. The carbon-hydrogen-oxygen balances for these analyses indicate the separation of carbon; it is to this, no doubt, that the condensates owe their color. Precise information as to the part played by the solid constituent of the condensate, which by analogy with the case of formaldehyde is the actual intermediate compound in the decomposition process, was not forthcoming, since sufficient of the solid was not available to permit of purification and subsequent bombardment. It was found, however, that on the window, where it was subjected to the more intense action of the rays, the condensate was highly viscous and contained a large amount of carbon, showing that decomposition of the polymer does indeed occur.

The effect of replacing one or both of the hydrogen atoms in the formaldehyde molecule with methyl groups may be observed in the analyses resulting from acetaldehyde and acetone given in Table II. The introduction of the second carbon atom has resulted in the appearance of small quantities of unsaturated hydrocarbons. The ratio of CO to CO_2 is increased. The ethane present in aldehyde mixtures is only a little above that which might be expected from the action of the rays on the methane present (8). On the other hand, the mixtures resulting from acetone have a high ethane content. This shows not only that two methyl groups on the same carbon atom can combine with the formation of ethane but also that the larger proportion of the methane formed in the acetaldehyde experiments results from the combination of the aldehydic hydrogen and the methyl group. The conclusion may also be drawn that the methyl groups are more remote in the intermediate complex formed during the decomposition of the aldehyde than in acetone; this is supported by the structures of the various polymers of acetaldehyde. But whether acetone first loses $=CH_2$ groups, thereby being capable of forming the same intermediate compound as acetaldehyde with the subsequent combination and hydrogenation of these groups to ethane, or undergoes decomposition by two simultaneous processes cannot at present be settled.

A comparison is made in Table VI of the compositions of the gaseous mixtures formed during the decompositions of methyl and ethyl alcohols. The outstanding point of these analyses is the high proportion of hydrogen. The results of Table VII establish the fact that after an initial change no appreciable accumulation of either formaldehyde or acetaldehyde occurs within the reaction chamber during bombardment. Consequently these substances must be continually decomposing with the formation of further products. Since the experiments of Series II and III show that formaldehyde decomposes more rapidly than acetaldehyde, a greater proportion of the decomposition must occur through the former than through the latter in the case of methyl alcohol. The formation of acetaldehyde during this bombardment must be due to the intermediate condensation of two molecules of the alcohol. With ethyl alcohol the tendency to decompose through formaldehyde is less; consequently, there

is the greater probability of the separation of the acetaldehyde polymer, a fact experimentally observed.

An important feature throughout all these experiments is the low unsaturated hydrocarbon content of the final gases. Indeed, the amount is barely measurable in the cases where the initial molecule contains less than two carbon atoms. As dehydrogenation reactions are promoted with the alcohols, the formation of ketene from acetaldehyde or acetone might be suspected. No trace of acetic acid could, however, be detected after passing the residual gases from the experiments in Series III and IV through water. The absence of higher unsaturated hydrocarbons eliminates any possible explanation of the high carbon dioxide contents by a reaction between ketene and acetaldehyde. Moreover, the low ethylene contents suggest that no ketene is formed since it is unlikely that it would be more stable to cathode rays than were the other substances examined. On the other hand, this almost complete absence of unsaturated hydrocarbons may be due to the fact that they are capable of reacting under the influence of high-speed electrons with the other substances present. But if this were so, then in view of the results obtained with acetone, higher ethane contents would be expected, particularly in the case of ethyl alcohol.

On account of the above facts, the experiments were extended in Series VII to an investigation of the behavior of ethylene under the action of high-speed electrons. Methane reacts only very slowly under the influence of cathode rays, giving small percentages of hydrogen and ethane (8); acetylene is polymerized with the formation of negligible quantities of hydrogen, the solid polymer being unchanged beneath the action of the rays (10). The reaction of ethylene stands between those of methane and acetylene since the oil formed by polymerization, or perhaps condensation, is decomposed on further bombardment into hydrogen, saturated hydrocarbons and acetylene. The possibility of the hydrogenation of the ethylene was not fully exploited. The facts established in the experiments of this series, together with the almost complete absence of unsaturated hydrocarbons in those of Series V and VI, indicate that, in the primary mechanism of the decomposition of the alcohols, a dehydration process can play only a minor part in comparison with one of dehydrogenation.

Summary

(i) Cathode rays rapidly polymerize gaseous formaldehyde and also decompose the resulting solid paraformaldehyde into hydrogen, methane and oxides of carbon. The latter mechanism is an intricate one, involving the breaking up of molecular complexes to yield simple gaseous products.

(ii) Yellow condensates are produced on exposing acetaldehyde and acetone to the action of the rays. These condensates are considered to be solutions of a white polymer of acetaldehyde in liquids which consist almost entirely of the original substance.

(iii) Gaseous mixtures are obtained after bombarding acetaldehyde and acetone. A study of the kinetics of the ultimate decomposition of acetaldehyde

at pressures below 40 mm. shows the reaction to be of zero order unretarded by the resultant products. It is concluded that these gases result from the decomposition of the polymerized aldehyde.

(iv) Cathode rays decompose methyl and ethyl alcohols with but the slightest separation of liquid products in the latter case and none in the former. The amounts of aldehydes present during the bombardment were determined and it has been suggested that the decomposition process consists of dehydrogenation with the subsequent decomposition of the aldehydes.

(v) A yellow condensate is produced on bombarding ethylene with high-speed electrons. This resembles an unsaturated hydrocarbon in its properties and yields hydrogen, acetylene and saturated hydrocarbons on further bombardment.

(vi) It would seem that, in general, the above decompositions occur as condensations. Molecular clusters are first formed under the influence of the rays, and these subsequently set free the simpler gases in an effort to produce more stable configurations.

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HUMIDITY MEASUREMENTS IN THE SLIP STREAM OF FLYING AIRCRAFT[1]

By D. C. Rose[2]

Abstract

This paper is a report on some experimental work on hair hygrometers and the wet and dry bulb thermometer type of hygrometer used in aircraft work. The results of preliminary observations using such instruments are given, the results indicating that hair hygrometers are not satisfactory. The findings obtained can be correlated easily with the weather conditions under which the flights were taken.

In connection with some experiments on the elimination of electrostatic charging of films in cameras used for aerial photography, it was found desirable to obtain information on the relative humidity and state of ionization in the slip stream of flying aircraft where the camera is usually operated. This paper is a report of relative humidity measurements of a preliminary nature taken during four flights on Jan. 8, March 2, April 18 and June 17, 1931, each flight lasting about two and one-half to three hours.

Instruments Used

Of the several known methods of measuring relative humidity, the wet and dry bulb thermometer type of hygrometer, in spite of its many disadvantages, seemed the most suitable for the measurements required. Hair hygrometers were also used but were found to be unreliable at reduced pressures and temperatures, and chemical methods did not seem applicable. Dew point hygrometers were not tried because of the difficulty in observation and the low temperatures involved. Griffiths and Awbery (1) tried an hygrometer which depended on the measurement of refractive index of a glycerine and water mixture. It might be suitable for ordinary aeroplane use, but did not seem to give any satisfactory results at low temperatures.

The wet and dry bulb thermometers were placed side by side in a copper case with open ends in the air stream. The wet bulb was kept moist by a wick dipped in a water reservoir in the usual way. This arrangement has two great disadvantages. In the first place, in the necessarily low temperatures (the lowest encountered was $-18°$ C.), the wick freezes causing the supply of fresh water to the wet bulb to cease. Having this point in view and considering the high velocity of air about the wick, a thick coarse wick was used rather than the usual close fitting type. (See discussion of this point below.) Another difficulty involved was the reading of the thermometers. At temperatures ranging from $10°$ to about $-15°$ C. the difference in temperature between wet and dry bulb thermometers is so small that precise thermometers having scale divisions not greater than a fifth of a degree, or at the most a half, should be used so that tenths of a degree could be estimated. In the

[1] *Manuscript received August 26, 1931.*
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case of liquid-in-glass instruments, thermometers with a fine bore are therefore required. For the first two flights mercury-in-glass thermometers were used having every tenth degree marked. It was found impossible to read these with any certainty, since the thermometers must be placed outside the cabin in the slip stream. A different system of measuring the temperature was therefore devised.

Instead of using mercury thermometers, a set of resistance thermometers was constructed, connected to a special bridge circuit, by means of which the temperature difference between the wet and dry thermometer could be calculated easily from the readings on a slide wire scale. A detailed description of this instrument has been published elsewhere (4). With the circuit used, the dry bulb temperature must still be read by a mercury thermometer. In the later flights the system of reading the mercury thermometers was greatly improved and instruments marked very clearly in half-degrees were used, so that it became possible to obtain comparative measures of the relative humidity with both the resistance and mercury thermometers.

The resistance thermometers were in oil-filled glass tubes having dimensions of about one-half inch by two inches. The wet bulb was covered with a thick layer of cotton and was saturated by pouring water on it about every 15 min. The thermometers were placed directly in the slip stream of the aircraft, being suspended with the other apparatus through the camera hole in a cabin monoplane.

The questions of wetting the wick, and the nature and surface of the wick are of extreme importance if precise results are to be obtained. This subject has been investigated by others (3) and it has been found that for exact results the wet bulb covering should be thin and smooth. In the present experiments the conditions were rather different, in that the temperatures were mostly below freezing. Hence the wet bulb covering was made thick so that it would hold a good layer of ice. This point will be mentioned again when the results of measurements taken with the resistance and mercury thermometers are compared. This cover as used gave quite consistent results when compared with other instruments in the laboratory.

Another problem of importance is the velocity of the air stream past the thermometers. Paine (3) gives upper and lower limits of 15 and 3 metres per second. The upper limit is due to the air at high speeds carrying away the water in spray from the wet bulb. This should have no effect in the present case, as the wet bulb was frozen most of the time. The velocity was about 30 metres per second. The reduced pressure due to altitude would also reduce the effect of too high a velocity. Paine's work was done at room temperatures and ground pressure.

The translation of the thermometer readings to relative humidity in per cent is also an important point. As far as the author is aware, there are no psychrometric tables available for readings at reduced pressures, hence a formula had to be used. Although many formulas for relative humidity have been developed, the following one (2, p. 15) seems most applicable to the present observations:

$$e = e' - 0.00066B(t - t')(1 + 0.00115t'), \tag{1}$$

where e is the vapor pressure; e', the saturation vapor pressure at the wet bulb temperature, t'; and B, the barometric pressure in mm. of mercury; the temperatures being in degrees centigrade. The term $(1+0.00115t')$ was omitted in the present calculations because it made a negligible difference in the temperature range involved.

An "Edney" hair hygrometer was also carried during the flights—being suspended just outside the camera hole in the slip stream. The hair hygrometer was calibrated at one point in the laboratory before each flight. It is hardly expected that a hair hygrometer scale designed to work at room temperatures and ground pressures would give satisfactory results at reduced temperature and pressure. The results indicate, as was expected, that the hair hygrometer is not very good for use in aircraft.

Results

First Flight

Fig. 1 shows a summary of the results of the first preliminary flight. Both the first and second flights should be considered only as tests of the apparatus, it being understood that the results are somewhat incomplete. Owing to the way in which the readings were taken the results show up better if the observations are plotted on a time scale instead of on altitude. The pressure was read on a Tycos barometer, reading in millibars, (Curve A) carried with the other equipment, and also on the altimeter on the instrument board of the aeroplane (Curve B). The barometer readings were not converted to altitude readings. The resistance thermometers were not taken on this flight. The wet and dry bulb thermometer readings from which the relative humidity (Curve D) was obtained were unsatisfactory. The thermometers were of very fine bore, graduated in tenths of degrees and were difficult to read. Also they were not of sufficiently low range (−12° C.), so for the higher altitudes no reading could be obtained. In fact, even at

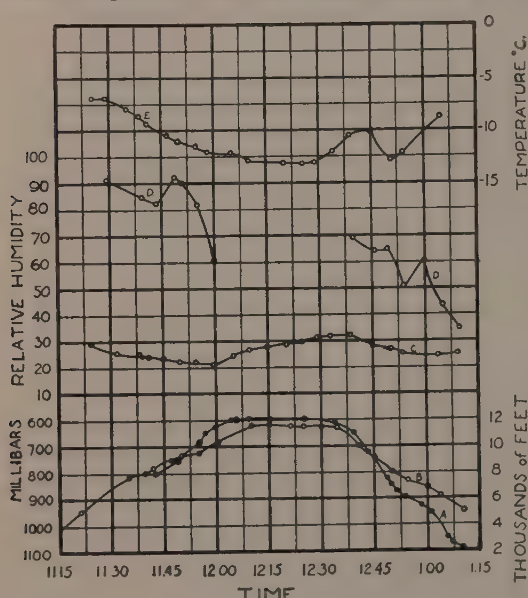

FIG. 1. *Curves representing results of the first flight. Curve A—Aircraft altimeter readings. Curve B—barometer readings. Curve C—Hair hygrometer readings. Curve D—Relative humidity from wet and dry bulb mercury thermometers. Curve E—Temperature from strut thermometer.*

the lower altitudes, owing to the difficulties in reading, the results are not very reliable. Curve C represents the readings of the hair hygrometer and *E* the strut thermometer attached to one of the wing struts of the plane. No exact record of the weather conditions of this and the third flight were taken at the time, but the day was clear and cloudless.

Second Flight

Fig. 2 shows a similar summary of the results of the second flight, which was made on March 2. This was the first flight on which the resistance thermometers were taken.

On this occasion the wet and dry bulb thermometers were not readable at all as the temperature was too low, but some results were obtained with the resistance thermometers. Curves A and B are the barometer and altimeter readings as in Fig. 1. It will be noted that in this and subsequent flights readings were taken at definite altitudes only, instead of during a continuous climb or descent as in Fig. 1. Owing to some trouble with the other apparatus used to measure ionization (not being reported here) the author was too much occupied to take many readings—hence the Curve C is not a complete curve but simply the humidity at the altitudes indicated. It will be noted that the second humidity observation (at 5000 ft.) is much higher than any other made during the flight. At first this was thought to be spurious but when the weather was considered, it is as would be expected. Just before the take off there was a light fall of snow and the sky was nearly overcast but clearing slowly. During the ascent there was quite a layer of clouds at altitudes around 3000 to 4000 ft. This probably accounts for the high humidity at 5000 ft. During descent most of the cloud bank was gone and, in fact, the descent took place over a considerable area which was entirely free from clouds, so no such increase in relative humidity was noticed. This increase in relative humidity due to the clouds was not noticed on the hair hygrometer (Curve D). Curve E is the temperature as measured by a strut thermometer.

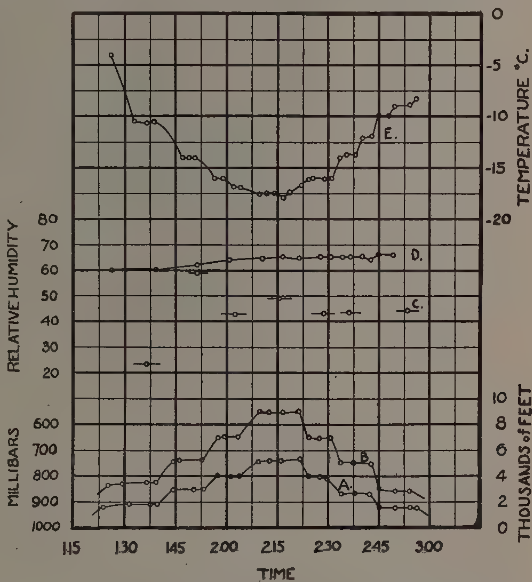

FIG. 2. *Curves representing results of the second flight. Curve A—barometer readings. Curve B—Aircraft altimeter readings. Curve C—Relative humidity from wet and dry resistance thermometers. Curve D—Hair hygrometer readings. Curve E—Temperature from strut thermometer.*

Third Flight

Fig. 3 represents the results of the third flight on April 18. For this flight a new set of mercury thermometers had been obtained, having every half-degree clearly marked and of lower range so that they could be read easily. Following along the time scale as one ascends, the temperature drops. The total moisture content of the air also drops, but relatively not as fast as the temperature, so the relative humidity increases to nearly 100% (Curve C). Then, ascending further, the temperature does not drop as rapidly and, since the moisture content is still decreasing, the relative humidity decreases. During the descent the process is reversed. No exact record of the weather was kept but it was either a clear or nearly clear sky, with a few fairly low cumulus clouds. The air was very bumpy at altitudes up to 4000 ft., making it difficult to take readings. The point marked X on Curve D is probably spurious as the water on the wet bulb thermometer was just freezing, making this thermometer temperature reading higher than it should be. This trouble always occurs near the freezing point when wet and dry bulb thermometers are used. The fact that the mercury thermometers (see Curve D) did not give the same results as the resistance thermometers is obviously due to unsatisfactory wetting of the bulb of the former, due to the freezing of the wick as previously explained. The wet resistance thermometer was saturated at every 2000-ft. level during the ascent, but as the supply of water was accidentally spilled when at the greatest height, it was not wet during descent. The consequence is that the humidity peak on descent is not as pronounced as during ascent. Care was taken not to take any readings immediately after wetting the wet thermometer, so that the temperature would have time to come to equilibrium.

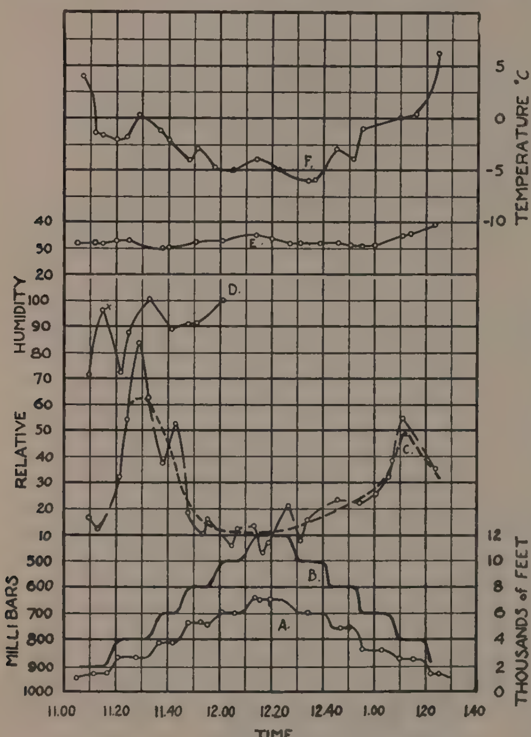

FIG. 3. *Curves representing results of the third flight. Curves A, B and C—as in Fig. 2. Curve D—Relative humidity from wet and dry mercury thermometers. Curve E—Hair hygrometer readings. Curve F—Temperature from dry mercury thermometer.*

The broken curve represents a probable average smooth curve, the sudden variations which are due to local air currents being omitted. One would expect the humidity curve to have variations in a reverse direction from those in the temperature curve, that is, as the temperature drops, relative humidity should rise and *vice versa*. To some extent this is so and a closer agreement would probably have been obtained were it not for the fact that the resistance thermometers show a lag greater by a few minutes than that for the dry mercury thermometer.

Curve E represents the readings of the hair hygrometer. It does not show the variations in humidity in agreement with the other instruments. Curve F shows the temperature read on the dry bulb thermometer in the slip stream.

Fourth Flight

Fig. 4 represents the results of the fourth flight made on June 17. The results obtained during this flight are more complete than any other. Readings were obtained throughout the flight of wet and dry bulb temperatures, both with the resistance and mercury thermometers. Curves A and B as usual represent the barometer and aircraft altimeter readings. In this flight, an altitude of 15,000 ft. was reached, whereas previously 13,000 ft. was the highest.

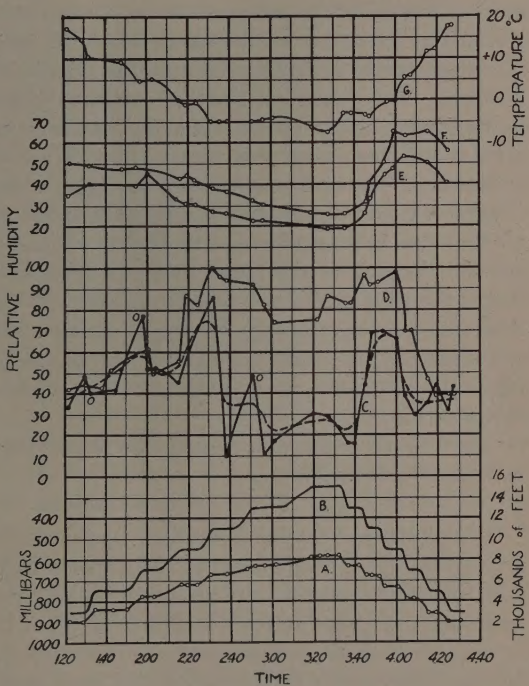

FIG. 4. *Curves representing results of the fourth flight. Curves A, B and C—as in Fig. 2. Curve D—Relative humidity from wet and dry mercury thermometers. Curve E—Hair hygrometer (same instrument as used in Flights 1, 2 and 3). Curve F—Hair hygrometer (belonging to Department of National Defence). Curve G—Temperature from dry mercury thermometer.*

Following the course of this flight: as the temperature dropped, the relative humidity (see Curve C) rose, showing two maxima—one small peak around 7,000 ft. and a large one at 9,000 and 11,000 ft. The relative humidity was rising while at the 9,000-ft. level and descending rapidly while at the 11,000-ft. level. The smaller peak around 7,000 ft. appears because of a drop in relative humidity while at the 7,000-ft. level, corresponding to a slight reversal in the temperature curve.

The curves representing relative humidity as measured with the wet and dry

bulb mercury thermometers (Curve D) followed closely results derived from measurements with resistance thermometers (Curve C) until about the freezing point, when the mercury thermometers indicate a higher humidity due no doubt to inadequate wetting of the wet bulb by the wick and reservoir method.

The relative humidity at altitudes higher than 11,000 ft. goes down considerably. Then during descent the process is reversed—a large increase in relative humidity appearing at the 9,000- and 11,000-ft. levels. This is exactly the sort of curve one would expect when the weather is considered. The day was clear except for numerous cumulus clouds at altitudes between 7,000 and 9,000 ft. The points marked "O" on Curve C are not as reliable as the others, as they were taken either when the wet bulb was just freezing or too soon after it had been saturated with water. They are probably higher than they should be. The broken curve represents an average smooth curve as in Fig. 3.

Curves E and F represent readings taken on hair hygrometers—E being read on the same hygrometer used in Flights 1, 2 and 3. An additional hair hygrometer belonging to the Department of National Defence was carried on this flight, the results being shown by Curve F. The relative humidity recorded from the hair hygrometer readings does not follow that calculated from the wet and dry bulb thermometer. On the descent an increase in relative humidity is shown corresponding to that shown on Curve C, but with considerable lag.

Curve G represents the temperature in the slip stream measured by the dry mercury thermometers.

Conclusions

The results indicate that the hair hygrometer is not satisfactory for use in aircraft work. Of the other two instruments used the resistance thermometers gave the better results, but no doubt equally good results could be obtained with mercury thermometers if an improved method of wetting the bulb were developed, and the thermometers so mounted that they could be easily read. In Fig. 4, the author has assumed that the resistance thermometers (Curve C) gave the more accurate results because the mercury thermometers had proved unsatisfactory in previous work.

The accuracy of the measurement is not very great and, though in fine weather the results indicate a low humidity (10 to 30%), at altitudes of 10,000 to 15,000 ft. there is a little uncertainty as to the correctness of this value because of the uncertainty in the accuracy with which the formula (1) can be used. Also, as stated above, the high velocity of ventilation and the rough wicks used may have some effect on the accuracy. However, owing to the consistent way in which the instrument behaved in the laboratory, it is not expected that the error is very great.

The experiments indicate that some rapidly responding, accurate and rugged form of instrument for measuring humidity is badly needed for aircraft work.

Relation to Weather

The results of these experiments, particularly the last two flights (Fig. 3 and 4), show definite agreement with what would be expected. As one ascends

to the cloud level, the relative humidity goes up nearly to 100%. One would expect that it should be 100% in, or in the neighborhood of, clouds. During these flights the aeroplane was not flown through or very near any clouds—which probably accounts for the fact that 100% humidities were not found except in cases where the readings of the thermometers were uncertain.

On a subsequent flight made by the author, carrying an aerometrograph only, which contains a hair hygrometer, the plane was flown through clouds for several minutes. The hair hygrometer showed an increase in humidity of about 15%, but not going above 60%. This is further evidence of the unsatisfactory nature of hair hygrometers for aircraft work.

Another interesting feature of these experiments was the altitude of the point at which maximum relative humidity occurred. In the two flights taken in cloudy weather (Fig. 2 and 4) the point of maximum relative humidity appeared to be a considerable distance above the clouds. This is particularly definite in Fig. 4. On this flight the clouds were between 7,000 and 9,000 ft. high, while the relative humidity maxima were found to be between 9,000 and 11,000 ft., both on ascending and on descending. In Fig. 2, the clouds were very low (about 3,000 ft.) and though the results here are not so complete, the maximum humidity recorded was at 5,000 ft. This may be due to the fact that during the flying, clouds were avoided as much as possible. This high recorded position of the point of maximum humidity, and the fact that 100% humidities were not recorded at all may possibly have been due to the location of the instruments in the aeroplane. It is quite possible that the air in the slip stream was slightly warmer than the atmosphere surrounding the plane because of heat from the engine. In fact in Flight 3 it was noticed that the temperature as read on the strut thermometer was about 2° lower than that read on the thermometer in the slip stream. This, however, was not the case in Flight 4, so it may have been due to the strut thermometer being inaccurate in Flight 3.

It is expected that further work of this nature will be undertaken by the author with improved equipment.

Acknowledgment

The author wishes to thank the officers of the Department of National Defence—particularly the pilots who undertook the flying—for their co-operation in this work.

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